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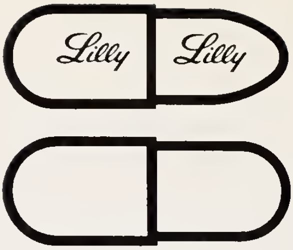

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Nineteen Fractures in an Accident Prone Patient: Psychiatric and Orthopedic Considerations

SAMUEL H. BASCH†, M.D., AND JOEL HARTLEY‡, M.D.

Although instances of self-inflicted injury are familiar to practicing physicians, the case of Mrs. A., a 69-year-old retired school teacher with a sequence of 19 fractures over a 35 year span, appears to be singular. She has been treated by one of the authors, Dr. Joel Hartley, for over 20 years. The majority of her fractures are well documented. Because of the impressive number of injuries, the patient has been evaluated for organic etiologic factors at several hospitals by numerous investigators. Studies for conditions such as hyperparathyroidism, osteogenesis imperfecta, osteomalacia, and osteoporosis produced consistently negative laboratory reports. Rapid healing followed each fracture.

Although superficially the patient appeared to be making an adjustment as a wife, mother, and school teacher, it was suspected by the authors that she was incapacitated in many ways by psychological conflicts. Between 1964 and 1968 these conflicts were investigated by means of periodic psychiatric consultations. Two psychological test batteries were also administered, although they will not be directly referred to in this report.

Brief Review of Literature

Previous attempts at understanding this type of behavior were made as far back as the late 1880's by Marbe, who was the first to describe non-chance accidents or accident habits (1). The incidence of multiple accidents in a small group of individuals was demonstrated statistically in the early 20th century (2) and many times thereafter. The unconscious aspects of accidental mishaps were referred to by Freud, who proposed that "falling, stumbling and slipping need not always be interpreted as purely accidental miscarriages of motor actions" (3). He referred to certain individuals having accidents as a result of an impulse which "takes ingenious advantage of an external situation that chance happens to offer, or, lends assistance to that situation until the desired injurious effect is brought about" (4). Accidents with distinct

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TABLE I

Fractures, Operations, Diagnoses, and Injuries Over a Period of 35 Years

Fracture Number	Age	Fractures, Operations, Diagnoses, and Injuries
1.	32	Fracture, closed left patella
	36	Hysterectomy
2.	49	Fracture, neck of left femur
	49	Traumatic synovitis, left knee
3.	50	Fracture, left ankle
4.	50	Fracture, metatarsal of the left foot
	52	Severe sprain, right ankle
5.	53	Colles' fracture, right wrist
	54	Severe contusions and strains
	56	First x-ray evidence of osteoporosis, 19 years post-hysterectomy
6.	56	Fracture, left olecranon
7. and 8.	56	Fracture, left lateral humeral condyle; fracture, tuberosity of the right humerus
9.	60	Fractured ribs; severe sprained right foot; low back pain
10., 11., and 12.	61	Fracture of the right ankle; fracture of the left humerus in two locations of its shaft
	62	Traumatic synovitis, left knee
13., 14., and 15.	64	Fracture, right radius; fracture, right ulna; fracture, neck of right femur
16.	65	Fracture, head and neck of right humerus; aseptic necrosis (avascular degeneration, right femoral head)
	66	Moore prosthetic replacement, head of right femur
17. and 18.	66	Fracture (comminuted, severe, and extensive), proximal two-thirds of left femur; fracture, neck of left humerus
19.	67	Fracture, side plate of Jewett nail associated with weak bony union of comminuted fracture, left femur

purpose, such as atonement for guilt, have been examined and discussed by Fenichel (5).

Case Report

DESCRIPTION OF PATIENT

Mrs. A. is a stout, well-groomed, well-rouged, flamboyantly dressed woman of average height. She has an air of gentility and, despite her physical incapacities, uses active and animated motions. Although intelligent, coherent, and able to complete individual thoughts, she is sporadically tangential and circumstantial. Her affect is appropriate but mildly labile.

PERSONAL HISTORY

The bulk of the anamnestic material was offered by the patient prior to the death of her husband in 1965. The material was presented to the orthopedic surgeon over more than 20 years, and to the psychiatrist during periodic interviews over a 4 year time span. Certain factors precluded the possibility of a sustained psychotherapy situation and, hence, no attempts were made to analyze the elements in depth. The data presented by the patient and the psychodynamics posed in this paper should be considered in this context. Despite the number of fractures, the patient was able to recall them clearly and report details quite consistently to both the orthopedic surgeon and the psychiatrist.

She describes herself as the only child in the family, born and raised in a "silk stocking" neighborhood. Her father, whom she "adored," was a butcher who was assisted by her mother at quartering meat. Her father also raised race horses which he himself helped groom and massage. In addition, he gave amateur dancing lessons.

Since the patient was a thin and fragile child, her father frequently massaged her legs with alcohol to strengthen them. This was soothing to her, and some of her fondest memories are of these gratifying moments following an insult or injury from her mother.

Mrs. A. recalls her father telling her, "You and I will live alone someday in a country home." During her early girlhood days, her father often kept her out late dancing which angered her mother. Her father had confided in her that he took her with him as protection, because otherwise the mother would accuse him of interest in other women. This type of seductive behavior on the father's part, using the patient as a pawn in the parents' struggle, was not atypical of her childhood. Her paternally influenced enjoyment in dancing persisted throughout her lifetime.

The patient claims that 1 year after her son's birth her father was "killed" by her mother, "who yelled so much he would tremble." One day after being screamed at by her he fainted, and Mrs. A. thought he had died. He did not die at that time, but did soon thereafter from nephritis.

The mother is portrayed by the patient as an inconsistent and contradictory person, strict, nagging, and unaffectionate, who never failed to share her physical complaints with the patient. The mother suffered from arthritis and diabetes; she had thin legs and weak ankles which she sprained frequently, and constantly complained of having "foot trouble."

The mother's overprotective attitude and constant feeding of "bone building tonics" served only to confuse the patient, who viewed her as overbearing and hostile. Only when the patient was ill would the mother show concern and affection.

The mother's hostility is depicted in an incident which occurred during the patient's earlier years. Her father had a cat which the mother habitually kicked. On one occasion the mother angrily hurled the cat, breaking its back, thus leading to its subsequent death.

The mother's overbearing attitude was reflected in her admonitions and modes of punishment. The patient's activities were restricted, and when she did go out the mother would warn, "Look out you don't get run over," or "Look out you don't fall." In retrospect, the patient construed these messages to be ambiguous and suggestive. Her mother would not allow her to coast on sleds or participate in other such activities. The patient would defy her and sneak off to climb on rocks and railroad tracks. The mother forbade the patient to use roller skates. When she discovered the patient using them, she placed the patient on the skates and pushed her over backwards to teach her a lesson. The patient hurt her spine. The mother would also punish the patient by throwing her down, sometimes in the bathtub, and by saying, "I'm going to kill you." Once, during a beating, she broke one of the patient's teeth with her wedding ring. Another frequent form of punishment imposed on the patient was beatings on the legs with a strap.

Conversation about, or allusion to, sex was forbidden. When she experienced menarche at age 13, she believed she was bleeding to death. Her mother instructed her that it was immoral to be touched by a man. Even after marriage, advice concerning sexual matters was continued by the mother.

In addition to her mother and father there were other family members who figured meaningfully in her earlier years. Her paternal grandfather favored the patient, who was similarly attached to him. She remembers his anger at her mother's beatings. The patient was 9 years old when he fell and broke two or three ribs. This accident greatly frightened her. The fall occurred on his way to bring his race horses indoors before they froze. The patient believed the ground was icy and dangerous, and so she tried to stop him by calling after him, "Don't go downstairs because everybody is falling." According to Mrs. A., he died 6 months later at age 74 from "sarcoma of the glands," which the patient attributed in some way to the fall and broken ribs.

An uncle died at age 33 from pneumonia and chicken pox, which he caught from one of his three children. The aunt and the three children then moved in with the patient's family. The patient blames her mother for her aunt's death several years later, which she felt was due to "aggravation." The aunt had warned the patient, "Your mother is like the cow who gives milk and then kicks over the pail."

Outside of her family, Mrs. A. had few friends or other relationships. She was fond of animals, as had been her father, and fantasied about them. She had daydreams of becoming a veterinarian and opening an animal hospital. At one time, her father had informed her that if horses fell, they had to be shot. Thereafter, she would cry whenever she saw an animal fall. She often likened herself to animals and recalls her mother dressing her in a manner which she describes as resembling "Astor's pet horse." She also spoke enviously of animals and often likened them to humans. Regarding her pet dog she stated, "That's the life. Everybody feeds him and pets him. My dog never really knows he's a dog, and thinks he's a person."

During her early school years she developed two fears: "Getting up on my feet and talking," and "falling." Since childhood she has experienced the repetitive dream of "falling into space," at times awakening in a panic. From an early age she also periodically dreamt of tragedies "befalling" her mother who died in 1946; since her husband's death in 1965, however, her husband has replaced her mother in the dreams.

When she entered adolescence she began dating despite her mother's discouragement. After several unhappy courtships, which she feels terminated because of her mother's interference, at age 22 she attended a dance and met her husband, who was 16 years her senior. Her husband, who was 44 at the time of their marriage, was strong, healthy, and prosperous. They often went dancing. She recalls their ice-skating together, and reflected on her unsteadiness, "It would take seven men to hold me up," a picture which resembled situations during some future fractures. Mrs. A. became pregnant early in the marriage, but her husband rarely was able to perform sexually during the remainder of their marriage, a fact that the patient bitterly resented.

Her husband had been in the wholesale shoe business when they married, but lost his money during the 1929 economic crisis. At that time the patient resentfully supported him by teaching and doing interior decorating. Otherwise, the patient reports that she "broke even" financially on the injuries by receiving health insurance, but passed up opportunities to sue because she feared going to court.

The patient became disillusioned and embittered as her husband aged and his material and physical resources waned. Arguments then became more frequent. On three occasions, in outbursts of anger, Mr. A. seriously directed the patient to jump out of the window.

Her husband developed diabetes in 1939, Parkinsonism in 1952, and Paget's disease of the bone in 1955. Inasmuch as he was physically ill, she was uncomfortable with any adulterous notions she entertained and reflected, "You shouldn't kick a man when he's down." At times she was aware of a great sexual drive, but would attempt to ignore it and avoid seeing men who were sexually attractive to her. On one occasion she asked her orthopedic surgeon if she fell because she was highly sexed. The discussion which follows shows sexual elements which appear involved in many of the circumstances surrounding her individual fractures.

She feels that she failed as a mother. She reports that because of financial burdens while she was pregnant her mother gave her ipecac, but the attempt at abortion was unsuccessful. She suffered a postpartum depression. She states that her father wanted her to have a daughter, and that both she and her father were disappointed that she had a son.

Prior to her son's puberty he had an undescended testicle, discovered by the patient's mother, for which he was treated with hormones. Although she dressed him up with pink ribbons as a baby, later she insisted he be taken to sports contests by his father so that he wouldn't be effeminate. By age 25 he had lived with several homosexual men.

ORTHOPEDIC HISTORY

The orthopedic surgeon, Dr. Joel Hartley, first saw Mrs. A. at age 49 following an injury in which she incurred a fracture of the neck of the left femur (see Table I for this and all subsequent fractures, and Plates I and II for representative x-rays of some of the fractures). Her past history revealed that despite numerous falls as a child, she had no fractures until age 32, when she suffered a fracture of the left patella, repaired by operation. Seventeen years later she fell and fractured the neck of the left femur, which was repaired by means of internal fixation with two Lippmann corkscrew bolts. Her recovery was prompt and excellent. Chemical studies at this time, and on several future occasions, revealed no evidence of a generalized bone disorder. In the same year as the above fracture, as a result of a fall, she developed a traumatic synovitis at the site of the former left patella fracture. This cleared up following aspiration. The following year she fell again, producing a fracture of the left lateral malleolus. This healed promptly after immobilization with a cast. During the same year torsion of her left foot in another fall resulted in a fracture of the metatarsal. This was successfully treated with a plaster cast. At age 52 she suffered a sprain of the right ankle from a fall. A year later she fell once more, incurring a Colles' fracture of the right radius which healed quite well with cast immobilization. The ensuing year she fell, experiencing severe contusions and strains of the body, but no fractures. During the next years she again fell on two occasions, causing contusions and sprains but no new fractures. At the age of 56, a repeat of the studies to rule out a general cause of bone disturbance was negative except for a mild postmenopausal osteoporosis, 19 years post-hysterectomy. No compressions of the vertebrae were noted during the subsequent 11 years of observation. In the same year, at the age of 56 years, she fell and acquired a displaced fracture of the left olecranon, which was corrected by internal fixation with a Sherman type screw. On the very day of the successful completion of this treatment, she left the office and fell in the street, sustaining a fracture of the left lateral humeral condyle, and a fracture of the tuberosity of the right humerus.

No new fractures were incurred during the next 4 years until age 60, when she fell, suffering a fracture of several right ribs, and a sprained right foot. In the same year she experienced low back pain. X-rays revealed the aforementioned mild osteoporosis and mild narrowing of the lumbosacral interspace associated with minimal osteophytosis. In the following year she fell on the street in Florence, Italy, and sustained three more fractures. The right ankle was fractured (the left ankle was fractured at the age of 50), as was the left humerus in two locations. She was treated with a walking short leg cast for the right ankle, and a plaster of paris-type Velpeau with a collar cuff sling for the left humerus. She healed readily once more. At the age of 62, she again fell, sustaining a traumatic synovitis of the left knee. This responded to aspiration and installation of corticosteroids. Two years later she developed an impacted fracture of the neck of the right femur, treated by means of internal fixation with a Lorenzo screw. At the same time, she suffered fractures of the distal one-third of the right radius and ulna, adjacent to the previous Colles' fracture incurred 11 years earlier. She recovered readily from these injuries, with malunion of the right radius, only to acquire a fracture of the head and neck of the right humerus after a fall. In the same year, at age 65, she experienced spontaneous insidious development of pain in the right hip, which proved to be avascular necrosis of the femoral head, 10 months after the internal fixation. This became progressively worse until the following year when the Lorenzo screw was removed and a Moore prosthesis was inserted (operation performed by Dr. Jacob Katz). Unfortunately, in the same year at age 66 she fell again, and this time sustained an extensive, severe comminuted fracture of the proximal third of the left femur, distal to the well fixed and undisturbed healed fracture of the neck of the femur. This was 17 years after the durable internal fixation of the neck of the femur with two Lippmann corkscrew bolts. A fracture of the tuberosity of the left humerus was sustained as a result of the same injury. At the operation the Lippmann corkscrew bolts were removed (operation performed by Dr. Jacob Katz), and internal fixation by means of a Jewett nail with a long side plate was performed. This fracture united, and she was able to walk well until a misstep, at age 67, caused a fracture of the side plate of the Jewett nail. A review of the previous roentgenograms indicated that indeed the bony union at the site of the refracture was not too solid, permitting undue strain and secondary fatigue fracture of the metal. Reoperation was not performed at this time, but she was kept at bedrest in simple traction. The fracture healed and she was able to walk again.

Thus, by the age of 67, over a period of 35 years, the patient sustained 19 fractures of the extremities. Repeated studies revealed no generalized osseous condition sufficient to encourage the occurrence of pathological fractures. In the latter 15 years of treatment, mild osteoporosis was identified, but not associated with any chemical changes. The osteoporosis, which appeared to play no important role in her fractures, was demonstrated 19 years posthysterectomy after she had incurred five fractures. All of the fractures resulted from falling injuries, and each time the treating orthopedist was satisfied that adequate external trauma had occurred.

DESCRIPTION OF ACCIDENTS

(*Correlation of Life Situation with Injuries*)

The circumstances surrounding each fracture were examined.

Fracture 1., 1931. This followed the discovery of her son's undescended testicle and the diagnosis of her husband's diabetes. It came after a year of crisis, during which time her husband rapidly lost his money.

One week prior to the accident the patient observed, in a rear view mirror, her husband attempting to kiss a friend's wife in a rumble seat. On leaving the car, her husband suddenly shifted the seat and banged her left knee, which infuriated her.

On the day of the accident, the patient felt guilty about attending a teacher's course and leaving her son at home sick with chicken pox, the disease from which her uncle had died. She was distracted by a movie theater billboard and, in a startled movement, stumbled over an obstacle, fracturing her patella. There were several young attractive men surrounding her who helped her up.

A 17 year interval elapsed between the first and second fractures. Although there were a number of minor injuries, no further fractures occurred until a few years after her mother's death. Immediately following the death, she had repeated minor falls and injuries which seemed to get progressively worse until the second fracture occurred. With these injuries and subsequent falls, she reported she often "knew" she might fall and have an accident, but felt "unable to prevent it."

Fracture 2., January 1948. The patient was leaving the same movie theater in front of which the first fracture had occurred, and the street was slippery. Her husband urged, "Don't run or you will fall." She ran and fell. She reported "It was icy and my legs spread. I slid and broke my hip. There were several young soldiers right there to help me."

The fracture occurred shortly after her son had moved in with another man, and she had become suspicious that he was a homosexual.

Fracture 3., April 1949; Fracture 4., May 1949. While her left ankle was healing from her April fracture, she visited friends at the beach. She was annoyed with her husband, who left her to play cards. She went walking on the boardwalk despite warning signs to proceed at one's own risk because of rotting boards. She stepped into a hole and fractured her left metatarsal. Three friends picked her up.

Her first four injuries all occurred to various parts of her left leg. Preceding her fifth fracture, her right ankle became strained. This injury, the first on her right side, occurred in early November, the time of year of at least four of her injuries, and of her postpartum depression.

Fracture 5., February 4, 1952. This right wrist fracture was the first upper limb injury. It occurred soon after her husband developed a pill-rolling tremor of his hand from Parkinson's disease. Her realization of his age at this time made her anxious, and his infirmity created in her feelings of resentment. From this point on, neither the upper nor lower limbs showed a preponderance of injury, nor was there a preference for left- or right-sided limbs.

Fracture 6., August 26, 1955. This followed the diagnosis of Paget's disease of the bone in her husband. She resentfully went to a job interview for him, and reported, "I was delayed in the interview at the Astor Hotel and since I was late to meet my husband I ran so that he wouldn't be angry, and I tripped on a step in the lobby and broke my elbow."

Fractures 7. and 8., November 11, 1955. Because her orthopedic surgeon, Dr. Joel Hartley, had been on vacation, the patient was being seen by his associate. On her last visit to the associate for the previous fracture, she was surprised to see Dr. Hartley back unexpectedly from vacation. She felt rejected and sorry that he was not examining her. Waiting caused her to feel annoyed, and a discussion regarding her return to teaching created anxiety. She left the office and went for an ice cream soda "as a tonic." After the "tonic," as she went to get a taxi, she tripped and toppled to the ground next to a man who helped her into a cab to go back to the doctor's office, where fractures of both upper extremities were diagnosed. She reported being relieved to be back in her doctor's care.

Fracture 9., 1959. The patient stated that she greatly resented having to entertain certain guests on her birthday. She was late in her preparations, which made her anxious. One guest sent a gift of flowers. While scurrying about and simultaneously fixing the flowers and preparing the meal, she dropped the flower vase and slipped in the water, falling and fracturing her ribs.

Fractures 10., 11., and 12., July 31, 1960. The patient traveled to Europe without her husband to take a teacher's course at a foreign university. While in Florence, Italy, standing in front of a jewelry store, she heard "men's voices, fun, and hilarity coming from a sidewalk cafe." She hurriedly headed there but tripped on the curb and fell, incurring the fractures. She screamed and several men nearby lifted her and rushed her to an orthopedic hospital, where diagnoses were made of a right ankle fracture and two left humerus fractures.

Fractures 13., 14., and 15., January 23, 1963. The patient was at home. Her husband was playing bridge with his friends, and she felt ignored and angry. She raced into the bedroom, slipped, and slid across the room, sustaining the three fractures of her right radius, right ulna, and right femur.

Fracture 16., March 5, 1964. After receiving an eviction notice on her first day out following an episode of pneumonia, the patient and her husband went to see their lawyer who angered them by keeping them waiting a long time. After leaving the lawyer's office the husband realized that they were standing on the wrong corner for the bus, and ordered the patient, "Run up there and get the bus." She began to run, slipped, and broke the head and neck of her right humerus.

Fractures 17. and 18., 1965. The apartment she had lived in for 28 years became a cooperative, and thus too costly, causing her to seek an inexpensive hotel to live in. She had hired a nurse to care for her, following difficulty with an orthopedic metal pin. The fractures occurred 2 days before the nurse was scheduled to terminate service, at which time the nurse accompanied her to see a hotel. The patient asked the nurse not to take the dog along because she needed "full attention." Despite the patient's protests, the nurse brought the dog and was minding it when the unattended patient slipped on the ice in front of the hotel she was considering, with the resultant fractures of her left femur and left humerus.

Fracture 19., 1966. The side plate of a Jewett nail failed, resulting in a fracture.

The orthopedic surgeon noted that following each accident the patient was talkative and almost euphoric. She appeared to derive satisfaction from being helped and demanding attention, and referred to her hospitalizations and contacts with her doctor as pleasant experiences.

DISCUSSION OF PSYCHIATRIC ELEMENTS

Although the data presented is suggestive, caution must be exercised in proposing psychodynamic explanations since there are no means available for validation. However, despite the difficulties in postulating plausible reasons for Mrs. A.'s behavior, symptom specificity, and other complex problems, an attempt will be made to correlate the history with the clinical findings.

A discussion of the psychodynamics has as its basis the premise that the falls and fractures were too numerous, and the background data too compelling, to be purely coincidental. This necessitates an examination of how her early life and familial experiences affected her as an individual, and influenced her to utilize falls and fractures as a means of coping with overwhelming feelings and conflicts.

Her background underscores her parents' contributions to her extraordinary mode of functioning. She came to assume a role as a victim of her mother's violence, requiring rescue by her father. She felt her mother's intense anger in both severe remonstrances and physical beatings. Violence in the household was observed when her mother killed the cat, and furthermore, certain family deaths were viewed by the patient as killings perpetrated by the mother's lethal "aggravation." She witnessed her mother, as well as her father, butchering animals in their shop. The mother's administering of ipecac to the patient was a feticidal gesture. The only relief in this hostile, violent environment, was the relationship with her father, fulfilling some of the dependency needs frustrated by her mother.

Guilt was engendered early by her mother, who imposed strict taboos on activity, motility, and sexuality. Intense resentment toward her mother was accompanied by guilt which was magnified because of her destructive wishes ultimately realized in her mother's death. Anxiety and guilt, resulting from sexual thoughts and impulses which occurred despite her mother's warnings, were underscored by the erotic gratification she received through her father's doting, and which she later sought through other men.

Her anxiety was not solely sexual, but the inevitable outcome of a mother, unpredictable and punitive, supplying overprotection and nutrient tonics one moment, and meting out severe punishment at another.

In each fracture, the patient appeared overwhelmed by anger, guilt, or anxiety, and by a need for relief from those disturbing feelings.

Her associations and identifications with animals in her earlier years figure in her later pathology. Just as she now invests her dog with human traits, she also invests humans, primarily herself, with nonhuman traits. This may have begun with her longing for the loving care which her father gave his cat, or her yearning for the concern and caressing attention he showed his race horses. However, enjoying these privileges was fraught with the danger of her mother's cat hurling wrath, or of being sacrificed for a fetlock injury, not to mention the position of the quartered creatures in the butcher shop. However, in her daydreams afflicted creatures could gain care in an animal hospital, in much the same way she herself could gain care in a hospital.

The selection of falling as a specific symptomatic act can also be seen as related to her earlier life experiences. Both her mother and her father had a peculiar investment in the limbs and in motility. The mother had weak ankles; although she was not affectionate toward her daughter, she expressed her maternal concern by giving her child bone strengthening tonics. Acts forbidden by the mother were mainly those of motility and action, the other prohibitions being related to sexual matters. Her mother's warnings about falling were ambiguously felt by the patient to be her mother's bidding. Of particular note were the methods of punishing the patient by pushing her over or strapping her legs. The patient appeared ultimately to take on some of her mother's traits of limb grievances, as well as developing destructive attitudes toward her own body, similar to those held by her mother and later held by her husband toward her.

The father's interest in limbs and motility was reflected in his occupational interest in horse racing. His avocation as a dancing teacher was shared with his daughter whom he seductively kept out late dancing. The father's role of massaging his daughter's legs to strengthen them, as he had done with his race horses, had a gratifying effect upon her.

Illness and death in her family are of significance and appear to have had a lasting effect on her. The patient's mother and husband both suffered from arthritis and diabetes. The death of her grandfather, uncle, aunt, and father remain vivid in her mind. The death of her mother is suspected of triggering her latter 18 fractures since she held ambivalent attitudes toward her. In addition, the onset of her husband's illnesses corresponded temporally with some of her fractures.

Her disturbed and extreme experiences in childhood did not lessen during her adult years since may of her conflicts were never resolved, and her adult life, in many ways, became a replica of her earlier days. Mrs. A. tried to simulate her relationship with her father by marrying an older man with an interest in dancing, and who exhibited other attributes similar to those of her father. It appears, however, that her husband ultimately turned out to be more like her mother. He developed arthritis and diabetes as her mother had, simulated the mother's concern for the patient during illness, and eventually even replaced Mrs. A's mother in dreams. Her husband's attitudes toward her appeared no less destructive than her mother's had been. This, added to Mrs. A.'s own internal self-destructive attitudes, consolidated her role as victim.

From childhood she harbored dependency longings for her mother's health tonics and the affection granted when an illness developed. Her need to be cared for with soothing relief was fostered by her father, although frustrated by her mother and later by her husband. Recumbent in her post-fracture hospital bed and in her doctor's hands, she may have achieved a dependency gratification which she could not have obtained in any other way.

From this background of anxiety and anger in her perceived role as a victim, the patient was confronted with situations which aroused previous conflicts. These situations were fraught with disorganizing anxiety, on some occasions sexual anxiety, and at times resentment. The sexual temptations, accompanied by guilt for harboring these desires, often precipitated her actions.

Focusing on her first two fractures in greater detail will help to illustrate specific dynamic factors which apply to the remaining fractures, and correlate her external reality situation with the internal overwhelming feelings of anger, anxiety, and guilt. It was not one individual conflict which precipitated the falls, but a constellation of complex factors which occurred with each fall.

The role of anger is predominant in the first fracture. Mrs. A. was infuriated at her husband's attempt to kiss his friend's wife, especially since the patient herself had frustrated adulterous desires. Vengeful thoughts toward her husband arose when he "accidentally" banged her knee, and the subsequent fracture may have represented an acting out on herself of her husband's aggression. In addition, she was disappointed that her husband had contracted diabetes since, as with her mother, it was necessary for the patient to be the more infirm of the two in order to achieve the greatly longed for care. His loss of money made him less of a provider which further disappointed her.

Guilt also played a role in the first fracture. The patient felt negligent leaving her son home with chicken pox and avoiding the disease which her uncle had lethally contracted from his own child. The feelings were compounded by the discovery of her son's undescended testicle, since she had attempted to feminize him. In addition, she felt a responsibility for her husband's diabetes, since she wished ill of him as she had her mother before.

With the underlying anger and guilt, the fall occurred at a moment of anxiety in the public setting in front of the theater and in the presence of young, attractive men, when her internal disequilibrium may have been overwhelming.

The second fracture took place 17 years later in the same theater setting as the first fracture, in the midst of young soldiers. Her sexual anxiety may have come forth, as well as sexual guilt, in the presence of her husband. By falling, she was able to both remain on the location with the soldiers to enjoy their attention, as well as to render herself incapable of undertaking any active contact with them and to readily accept her self-ordained punishment.

The role of guilt in the second fracture goes beyond the sexual considerations. Since the patient continued to harbor unresolved ambivalent feelings, conflict regarding her mother's death was a factor here and for all future accidents. The second fracture followed a series of minor mishaps which could have served as lesser attempts to punish herself for hostile feelings she had toward her mother. These minor accidents built up to a crescendo resulting in the left hip fracture. Her husband, just as her mother had done previously, had ambiguously instructed the patient not to run, lest she fall. The patient defied this command. Another element of guilt was felt by her when her son moved in with another man whom she suspected of being homosexual, and she realized her role in adversely influencing her son, just as her mother had destructively influenced her.

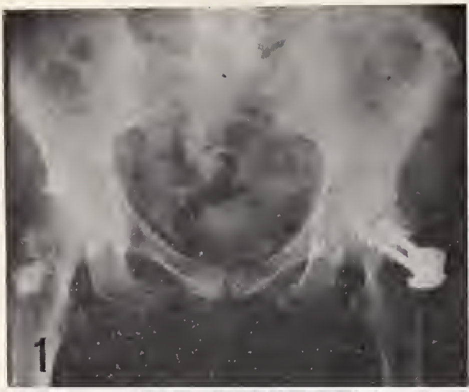
The first two fractures appear representative of her subsequent fractures, and the elements contained in her earlier accidents do not appear to differ significantly from the later ones.

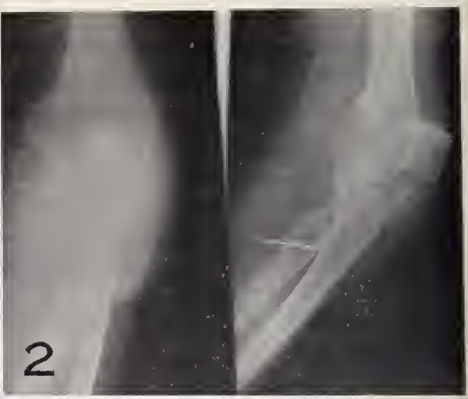
Summary

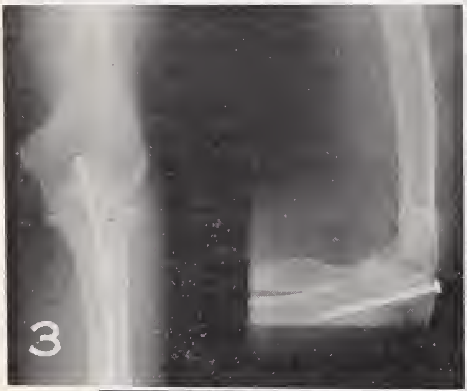
The historical data of a 69-year-old woman with 19 fractures and no metabolic disorder has been presented, and an attempt made to correlate the data with her current clinical orthopedic status. From early childhood the patient was influenced by an overprotective and sadistic mother. The satisfaction, not adequately derived from her mother's tonics and maternal concern when she fell ill, was sought from her seductive and attentive father.

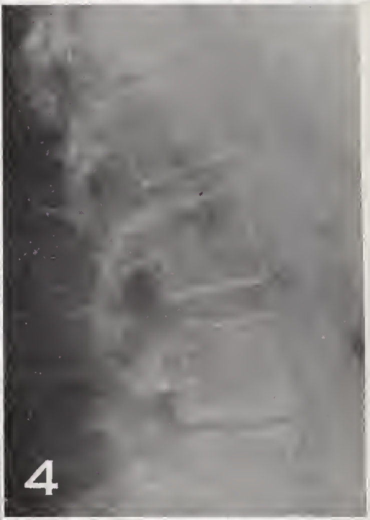
A strong family investment in motility, limbs, and animals were prominent as was the incidence of injury, illness, and death, all of which later influenced her mode of dealing with conflicts.

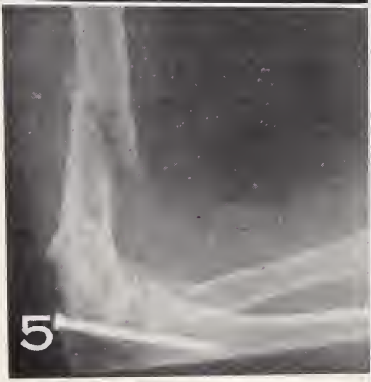
Her inability to cope with her own overwhelming feelings of sexual and other anxieties or anger, her difficulty in handling the intensely felt guilt, and her residual dependency longings, all led to acting out in a remarkable self-destructive manner.











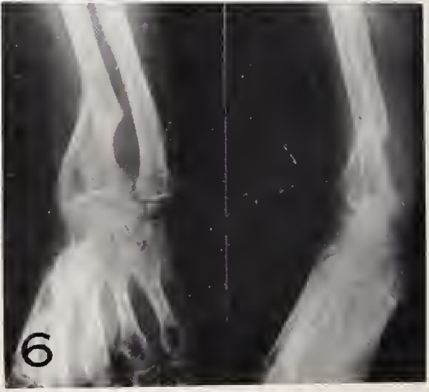


PLATE I

Fig. 1. Internal fixation of fracture of neck of left femur with two Lippmann cork screw bolts.

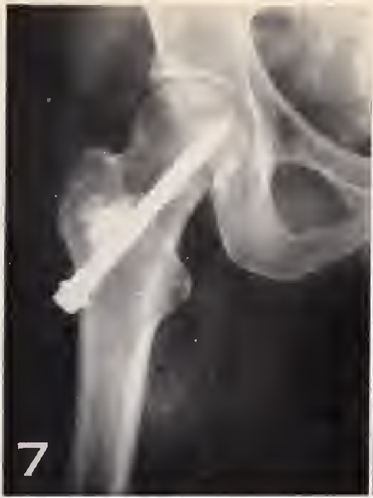
Fig. 2. Fracture of left olecranon.

Fig. 3. Internal fixation of fracture of left olecranon, with a long screw.

Fig. 4. First x-ray evidence of osteoporosis, 19 years post-hysterectomy. This never progressed to involve collapse of vertebral bodies.

Fig. 5. Fracture, distal one-third, left humerus lateral view.

Fig. 6. Fracture, distal one-third, right radius, healed with malunion.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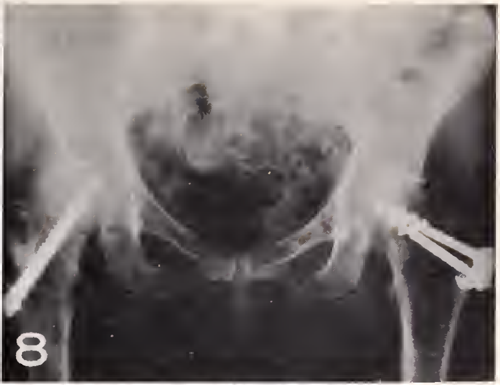
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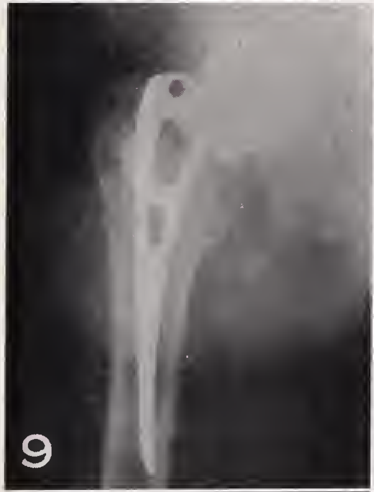
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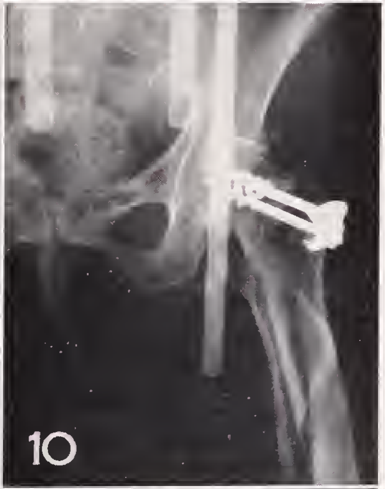
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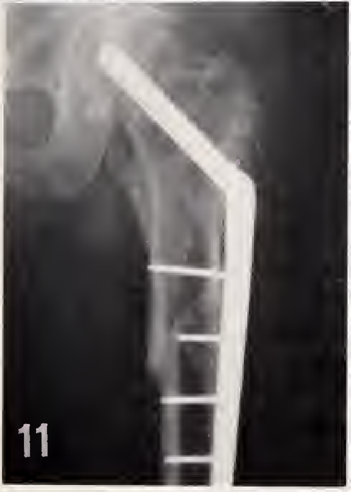
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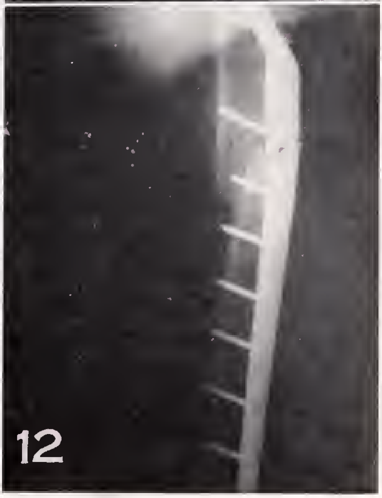
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PLATE II

Fig. 7. Internal fixation of a valgus impacted fracture of neck of right femur with Lorenzo screw.

Fig. 8. Delayed development of avascular necrosis, head of right femur, 10 months after internal fixation.

Fig. 9. Moore prosthetic replacement, head of right femur.

Fig. 10. Severe comminuted fracture, proximal one-third, left femur, 17 years after successful internal fixation of neck of femur with two Lippmann cork screw bolts.

Fig. 11. Internal fixation, left femur, seen in Figure 10 after removal of cork screw bolts.

Fig. 12. Fracture of side plate of Jewett nail of the left femur, 10 months after fixation as a result of a simple tripping type of injury.

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The Intensity of the First Heart Sound in the Wolff-Parkinson-White Syndrome*

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The first heart sound in the Wolff-Parkinson-White syndrome (WPW syndrome) is generally described as normal or diminished in intensity on the basis of clinical impression and a few published phonocardiograms (1–4). By employing atrial pacing techniques previously utilized in the study of the WPW syndrome (5), it was possible to obtain a controlled comparison of the first heart sound in three patients during both anomalous and normal conduction.

The provocative finding of an increased intensity of the first heart sound during anomalous conduction constitutes the substance of this report.

Methods

Four patients, with the WPW syndrome as their only manifestation of heart disease, had investigational studies. They were W.R., a 33-year-old male with a 6 month history of palpitations and constant type B anomalous conduction; B.H., a 68-year-old male with 20 years documentation of type A anomalous conduction; N.R., a 42-year-old female with a 2 year history of intermittent type A anomalous conduction; and L.R., a 35-year-old male with a recently observed type B anomalous conduction.

Each study was performed in the postabsorptive state and the supine position. A transvenous pacing catheter was introduced percutaneously into an antecubital vein and positioned under fluoroscopic control against the lateral wall of the right atrium. From this position, heart rate was controlled with a battery powered pacemaker (Medtronic "R" wave coupled pulse generator). Phonocardiograms of the mitral valve area were recorded simultaneously with multiple electrocardiographic leads on an Electronics for Medicine oscilloscope photographic recorder at paper speeds of 25, 50, and 100 mm/second. Phonocardiograms were recorded at a frequency between 400 and 2000 cycles/second

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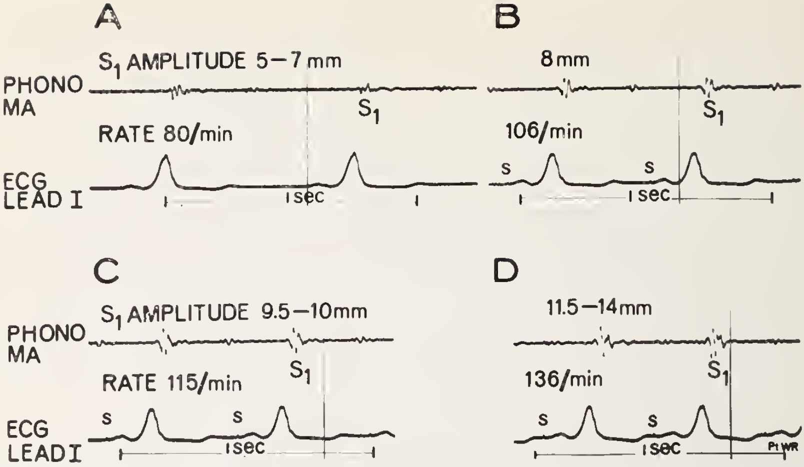

FIG. 1. *Increasing intensity of the first heart sound with faster heart rates during anomalous conduction.* Patient W.R. Progressive increase in heart rate from 80 beats/minute (A) to 136 beats/minute (D) is accompanied by an increased maximum wave deflection of the first heart sound (S_1) from 5–7 mm (A) to 11.5–14 mm (D).

with a standard bell shaped microphone. Neither microphone position nor recording sensitivity was altered after the commencement of a study.

The maximal wave deflection of the first heart sound was measured in millimeters and chosen as an expression of intensity (6–9). When variations in amplitude of the first sound occurred during the respiratory cycle, the range of variation was noted.

Atrioventricular conduction time was considered to be the interval from stimulus artifact to onset of R wave during atrial pacing.

Care was exercised to assure that all electrical apparatus was properly grounded and that the electrode terminals of the catheter were not handled by the operator during fluoroscopy.

Results

Each study was completed without complication. Each patient had a progressive rise in the amplitude of the first heart sound during anomalous conduction as heart rate was incrementally increased (Fig. 1).

Three of the four patients converted from anomalous to normal conduction during atrial pacing and, in doing so, provided data for a controlled comparison of the first heart sound during both anomalous and normal conduction. The results are summarized in Table I. In two instances, the intensity of the first heart sound was greater during anomalous conduction than during normal conduction (Fig. 2). In the remaining case, the first heart sound intensity was similar during both anomalous and normal conduction. Alternating beats of anomalous and normal conduction also occurred at a constant paced heart rate in patient N.R., with a greater intensity of the first sound during anomalous conduction (Fig. 3).

TABLE I

Patient	Rate/minute	Normal		Anomalous	
		S†-R/m sec†	S_1†amp†/mm	S-R/m sec	S_1 amp/mm
L.R.	100	160	8.5–9	72	8.5–9
B.H.	107	191	6–9	106	9–15
N.R.	90	190	6–8	103	8–10
W.R.*	120	230	7–9	110	26

* Alternating normal and anomalous conduction.

† S = stimulus artifact; m sec = millisecond; S_1 = first heart sound; amp = amplitude.

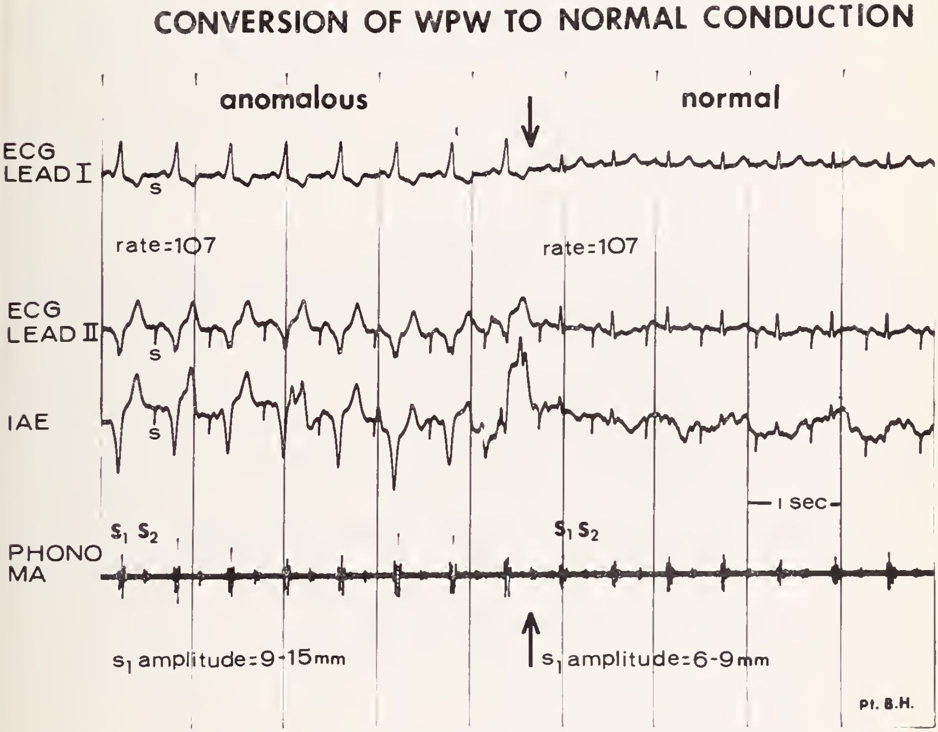

Fig. 2. *Conversion of anomalous to normal conduction.* Patient B.H. Continuous recording at heart rate of 107 beats/minute. Anomalous conduction precedes the arrows which mark the time of conversion to normal conduction. During anomalous conduction, the first heart sound (S_1) has a maximum amplitude wave deflection ranging from 9–15 mm. During normal conduction, the S_1 amplitude range is 6–9 mm. IAE = intra-atrial electrogram; Phono MA = phonocardiogram mitral area; S = pacemaker stimulus artifact; S_2 = second heart sound.

Arterioventricular conduction time remained less than 200 m sec during atrial pacing in all cases of normal ventricular depolarization, with the exception of a 230 m sec recording during the alternating normal conduction of patient N.R.

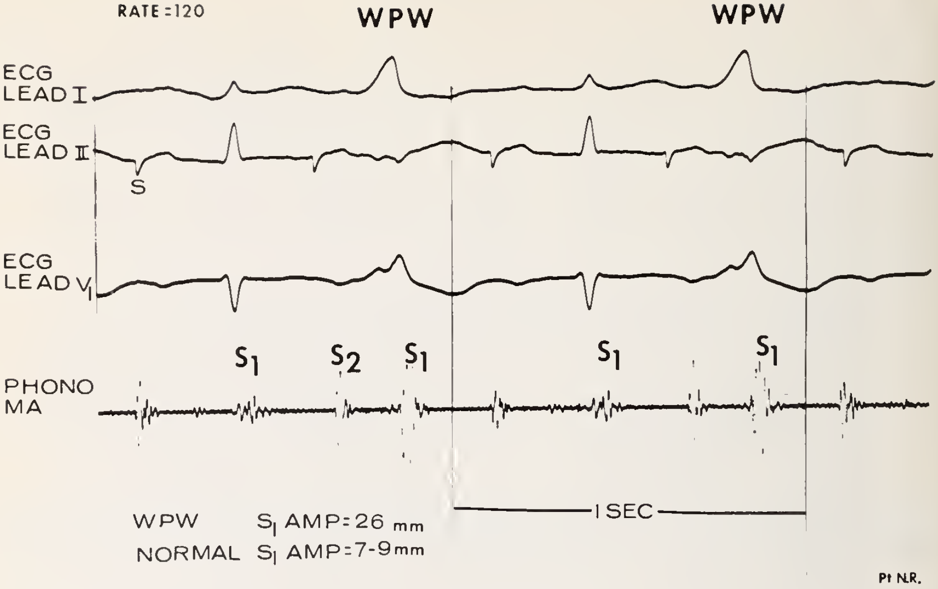

FIG. 3. *Alternating anomalous and normal conduction.* Patient N.R. Heart rate 120 beats/minute. Anomalous beats are labeled WPW and are accompanied by a first heart sound (S_1) with a maximum wave deflection of 26 mm. Normal beats have an S_1 amplitude range of 7–9 mm. Phono MA = phonocardiogram mitral area; S = pacemaker stimulus artifact; S_2 = second heart sound; AMP = amplitude.

Discussion

The intensity of the first heart sound is predominantly governed by the position of the atrioventricular valves (A-V valves) at the time of ventricular systole, and by the force of ventricular contraction (10). The position of the A-V valves at the time of ventricular contraction in turn is determined in part by the ventricular diastolic filling period (heart rate) (11–12), and in part by atrioventricular conduction time (P-R interval) (13). In the present study, a comparison of the first heart sound at a constant heart rate, during both anomalous and normal conduction, excluded heart rate *per se* from influencing the comparison. Atrioventricular conduction time, however, was always abnormally short during anomalous conduction (14).

Lown, Ganong, and Levine (2) contrast the loud first sound of the syndrome bearing their names (short P-R interval, normal QRS complex, and paroxysmal rapid heart action), with the normal or muffled first sound of the WPW syndrome in patients with heart rates of less than 100 beats/minute.

If the short P-R interval in anomalous conduction is reflected in early ventricular contraction, an increased intensity of the first heart sound would be expected. It appears that hemodynamic and kymographic study has yielded varying results in the timing of ventricular events during anomalous conduction. Ferrer and associates (15) studied electromechanical events in 2 patients with the WPW syndrome, and concluded that systole was delayed in both ventricles. March, Selzer, and Hultgren (16) reported electromechanical events in 12 patients with anomalous and normal conduction. They concluded that left ventricular ejection was premature in 2 cases, delayed in 3 cases, and was without abnormality in the remainder. Electrokymographic study of the great vessels by Samet, Mednick, and Schweidel (17), in 15 cases of the WPW syndrome, indicated delayed ventricular ejection. Bandiera and Antognetti (18), employing roentgenkymographic methods, demonstrated early excitation of a limited area situated in the left ventricle in type A anomalous conduction, and in the right ventricle in type B anomalous conduction.

The force, or tension, of ventricular contraction is the remaining variable effecting the first heart sound. Although several convincing experimental studies have stressed the importance of this variable as expressed by the first derivative of left ventricular pressure (19–20), no such measurements have been made during anomalous conduction in man.

It is concluded from data obtained during controlled heart rates that a louder than normal first heart sound may accompany anomalous conduction. The precise incidence and mechanism of occurrence must await future investigation.

Summary

Four patients with the Wolff-Parkinson-White syndrome had phonocardiographic determinations made of first heart sound intensity. During anomalous conduction, the first heart sound became more intense at progressively faster heart rates controlled by transvenous pacing of the right atrium. A comparison of the first heart sound was also obtained at a constant heart rate in three of the patients during both normal and anomalous conduction. In two of the three instances, the first heart sound which accompanied anomalous conduction had a greater maximum wave deflection on phonocardiogram. It is concluded that a louder than normal first heart sound may accompany anomalous conduction.

Acknowledgment

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Clostridium Welchii Brain Abscess following Craniotomy for Meningioma: A Case Report

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The patient, a 45-year-old taxicab driver, complained of headache and blurring of vision of 3 weeks duration. He had been well until he suffered a hematoma of his forehead during a head-on collision between automobiles. He did not lose consciousness but he was dazed. He began to complain of headache 5 or 6 hours after the accident. 48 hours later he consulted a physician because of increasingly severe headache and blurring of vision. Upon examination 3 weeks later there was slight incoordination of the left lower extremity. Early papilledema was noted. Visual acuity in the right eye was reduced to hand movements and counting fingers. The patient was admitted to the hospital. Except for an operation for gastric ulcer 4 years prior to this hospital admission, the patient had previously been in good health.

Neurological examination in the hospital was essentially as noted above. His memory was impaired. Electroencephalogram done the day after admission showed almost continuous 0.9–3/second activity in the right anterior temporal and right anterior frontal electrodes. X-rays of the skull showed increased vascularity in the diploë. Right carotid angiography showed marked displacement of the anterior cerebral artery. The frontopolar artery was of large caliber and displaced downwards. The internal cerebral vein was markedly displaced backwards. The findings indicated a large right frontal mass.

Six days after admission a right anterior frontal craniotomy was done and a large (9 x 7 x 5 cm, 137 gm) subfrontal meningioma was totally removed. The postoperative course was unremarkable except for fever of 101°F to 102.6°F which began on the first postoperative day. The wound seemed to be healing well and the scalp sutures were removed on the fourth postoperative day. On the 11th postoperative day a thin purulent drainage was noted from the stab wound, the site of a rubber drain kept in place for 24 hours after the craniotomy. On the 19th postoperative day there was a murky brown-red discharge from the wound. On culture Clostridium welchii was grown. Vigorous treatment with penicillin, Achromycin®, and sulfadiazine was started. Although the patient's general condition improved and his fever subsided, the wound continued to drain. Six weeks after the first operation the wound was reopened. The bone flap was removed. A sinus tract was found leading into a large abscess cavity which began at the frontal pole and extended 4 cm posteriorly. The wall of the abscess and surrounding softened brain were removed. The bone flap was not replaced. A large catheter was placed in the cavity remaining after the brain abscess removal. Intensive therapy with penicillin and sulfadiazine was continued. In addition, 20,000 units of penicillin was injected

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into the cavity daily through the catheter. Sixteen days later cultures from the cavity were negative. The catheter was removed from the cavity on the 20th postoperative day and replaced with a narrow packing which was then slowly removed over the next 12 days. The patient improved and he was sent home 35 days after the second craniotomy.

Microscopic study of the tumor removed at the first operation disclosed fairly cellular, moderately vascular tissue with areas of necrosis and hyalinization. The appearance was typical of a fibromatous meningioma.

The material removed at the second operation consisted of highly vascular granulation tissue with extensive inflammatory changes.

At home the patient made excellent progress. His mental status improved, as did his vision. Although he had been taking 100 mg of Dilantin® (diphenylhydantoin) twice daily since the first operation, about 10 months following the second operation he had a generalized seizure. Medication was increased. Nevertheless, the seizures recurred at irregular intervals.

In April of 1958 the patient was readmitted to the hospital for the repair of the skull defect. An EEG at this time showed a small amount of 1.5–3 cps activity in the right frontal, anterior, and midtemporal electrodes. The grand mal seizures continued in spite of large doses of various anticonvulsant medications. The patient was advised to reenter the hospital for further neurosurgical therapy for control of the seizures, but refused to do so.

Comment

Clostridial infection complicating surgical intervention for brain tumor is very uncommon and has not been reported before as far as we know. In all of the previously recorded cases of gas bacillus infection in the brain, a prior traumatic injury was involved. Bagley (1938) reviewed the literature and reported 2 cases. He was able to collect 21 cases up to that time, most of them due to penetrating wounds. In a report of 300 brain injuries as a result of shell and mine fragments in World War II, Russian investigators noted infection with gas producing anaerobes in 11.2%.

Dandy (1927) reported the case of a 6-year-old boy who developed marked increased intracranial pressure 1 week following an operation for mastoid disease. Trephine exploration was done and an abscess was found from which 30 cc of foul smelling pus was aspirated. One week later the abscess was tapped; again gas bubbled through the brain cannula. Unfortunately, the organism could not be identified.

Gilbert, Tolmach, and Farrell (1961) reported a case of gas gangrene of the brain following a compound depressed skull fracture. In their case, focal neurologic signs appeared 2 days after the injury. Debridement was done at that time. Bubbles of gas and necrotic brain exuded from the fracture site. No definite abscess capsule was found. Paracolobacterium and Clostridium perfringens were cultured from the abscess. Neomycin and bacitracin were used locally, and Chloromycetin® systemically. The patient recovered, although at the time of discharge from the hospital, 4 months after the injury, he still had residual left hemipareis.

A recent report by Russell and Taylor (1963) describes a patient who suffered a penetrating wound of the left temple by a rusty nail. Within a few days he began to have severe headache and his behavior was "odd." Eleven days after the injury he was drowsy, dehydrated, and disoriented. His temperature was 101.4°F. X-rays of the skull showed a gas bubble in the region of the left temporal lobe. Trephine exploration disclosed an abscess at a depth of 2 cm. The patient had a stormy course; however, following repeated aspirations of the abscess and full dosage of penicillin, sulphadimidine, and therapeutic doses of polyvalent anti-gas gangrene serum intravenously, he eventually made a full recovery. A pure growth of Clostridium welchii was isolated from the pus.

Russell and Taylor found 16 other cases of circumscribed gas gangrene abscess of the brain which were well documented and proven by positive culture during life. Their case was the 17th, and our case is the 18th.

This case is unique in that a well encapsulated Clostridium welchii abscess developed in the brain at the site of a tumor removal. We have not found a similar occurrence in a rather thorough perusal of the literature.

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Primary Amyloidosis Presenting as Malabsorption with Peripheral Neuropathy: A Case Report*

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Introduction

Primary amyloidosis is a systemic disease consisting of diffuse tissue infiltration of a protein-polysaccharide complex deposited in a fibrillar pattern (1). The pathogenesis of the malabsorption which occurs in some patients with primary amyloidosis is not well understood. This report describes a case of primary amyloidosis presenting with rapid weight loss, steatorrhea, peripheral neuropathy, and autonomic dysfunction. The diagnosis of amyloidosis was made by biopsies of the stomach, small bowel, and rectum. At autopsy there was diffuse organ infiltration with amyloid. Involvement of the autonomic ganglia with amyloid may have been important in the pathogenesis of the malabsorption syndrome. An interesting reduction in serum immunoglobulins of all groups was present as well.

Case Report

W.P., a 44-year-old white male, was in good health until August 1966, when he began having 3 to 4 watery loose stools per day without blood, pus, or mucus. This lasted for a period of 2 weeks and disappeared without treatment. During the next 6 months he experienced progressive anorexia and malaise, and lost approximately 50 pounds of weight. There was no nausea, vomiting, hematemesis, melena, abdominal pain, specific food intolerance, chills, or fever. In February 1967 he noted the rapid onset of explosive diarrhea consisting of 8 to 10 bulky, greasy, foul smelling stools per day, and the gradual onset of numbness and tingling in the distal extremities. He was admitted to a hospital and treated with parenteral fluids, vitamins, and tetracycline for "idiopathic diarrhea," and electroshock for depression without response. In April 1967 he developed postural hypotension in addition to the persistent diarrhea and paresthesias, and was admitted to another hospital. An upper gastrointestinal series showed barium retention in the stomach 7 hours after ingestion and moderate segmentation in the small bowel. The barium enema was normal, as was a small bowel biopsy stained with hematoxylin and eosin. High doses of prednisone, tetracycline, pancreatin, and vitamins were administered during the subsequent 3 months without improvement. The diarrhea, paresthesias, and postural hypotension continued, and impotence and urinary retention developed.

There was no family history of diabetes mellitus, hyperthyroidism, or gas-

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trointestinal disease. He smoked 1 to 2 packs of cigarettes per day for 25 years and drank 6 bottles of beer per week. He had not travelled outside the United States during the past 20 years. The past history was noncontributory.

Physical examination on September 14, 1967 revealed a thin, cachetic, lethargic, dehydrated white male appearing chronically ill. The pulse was 84 per minute and regular. The blood pressure was 90/60 supine, and 70/40 sitting. The temperature was 98°F. The pupils reacted equally to light and accommodation. The fundi were normal. The tongue was not enlarged but the papillae were atrophic. The lungs were clear to percussion and auscultation. The heart sounds were distant. There was no abdominal distension, tenderness to palpation, masses, or hepatosplenomegaly. The bowel sounds were hyperactive. There were no rectal masses but the anal sphincter tone was markedly decreased. There was extreme muscle wasting which was greatest in the extremities but there were no trophic ulcerations. There was no lymphadenopathy, cutaneous pigmentation, or dyshydrosis. Sensation to pin prick was decreased to absent in the distal third of the upper and lower extremities bilaterally. The deep tendon reflexes were absent in the lower extremities bilaterally and 1+ in the upper extremities. Muscle weakness was greatest in the distal extremities. The Babinski sign was flexor bilaterally.

The hemoglobin was 14.5 gm%, hematocrit 46%, white blood count 9,200/mm^3, with 73% segmented forms, 7% band forms, 14% lymphocytes, 2% eosinophils, 3% basophils, and 1% monocytes, and the Wintrobe sedimentation rate was 6 mm/hour; urinalysis: pH 5, specific gravity 1.005, albumin—trace, sugar—0, microscopic—normal; VDRL, negative; blood urea nitrogen 18 mg%, fasting blood sugar 80 mg%, carbon dioxide 26 mEq/L, chlorides 100 mEq/L, sodium 132 mEq/L, potassium 3.6 mEq/L, calcium 10.8 mg%, magnesium 2.6 mg%, uric acid 6.0 mg%, cholesterol 130 mg%, bilirubin (total) 0.5 mg%, SGOT 25 units, creatinine 0.9 mg%, prothrombin time 13/12 seconds; total protein 4.7 gm%, albumin 2.82 gm%, globulin: alpha$_1$ 0.22 gm%, alpha$_2$ 0.55 gm%, beta 0.44 gm%, gamma 0.69 gm%-IgG 580 mcg% (normal range 1200 ± 300), IgA 60 mcg% (normal range 280 ± 100), IgM 47 mcg% (normal range 88 ± 22). Stool: guaic reported as trace, ova and parasites were negative on 6 specimens. Serum carotene was 22 mcg%. Serum iron was 86 mcg%. Total iron binding capacity was 226 mcg%. Serum B$_{12}$ reported as 249 picograms/ml (normal range 200–900). Serum folate was greater than 300 nanograms/ml (normal range 7.0–15.9). Fecal fat measured 83 grams in 72 hours. Xylose absorption test showed 1.2 gm excreted 5 hours after ingestion of 25 grams. Intravenous glucose tolerance test was found normal. PBI was 4.6 mcg%. Urine arsenic reported negative. Urinary 17-OH steroids/24 hours reported as 14.6 mcg. The x-ray of the chest was normal. The intravenous pyelogram showed normal kidneys and an enlarged urinary bladder with retention. Upper gastrointestinal series showed delayed gastric emptying and slight segmentation and dilatation of the small bowel (Fig. 1). Barium enema was reported normal. The electrocardiogram showed right axis deviation, elevated ST in AVL, and V$_{3\text{-}6}$. These were interpreted as nonspecific changes which may be seen in chronic pulmonary disease. Motor nerve conduction velocities

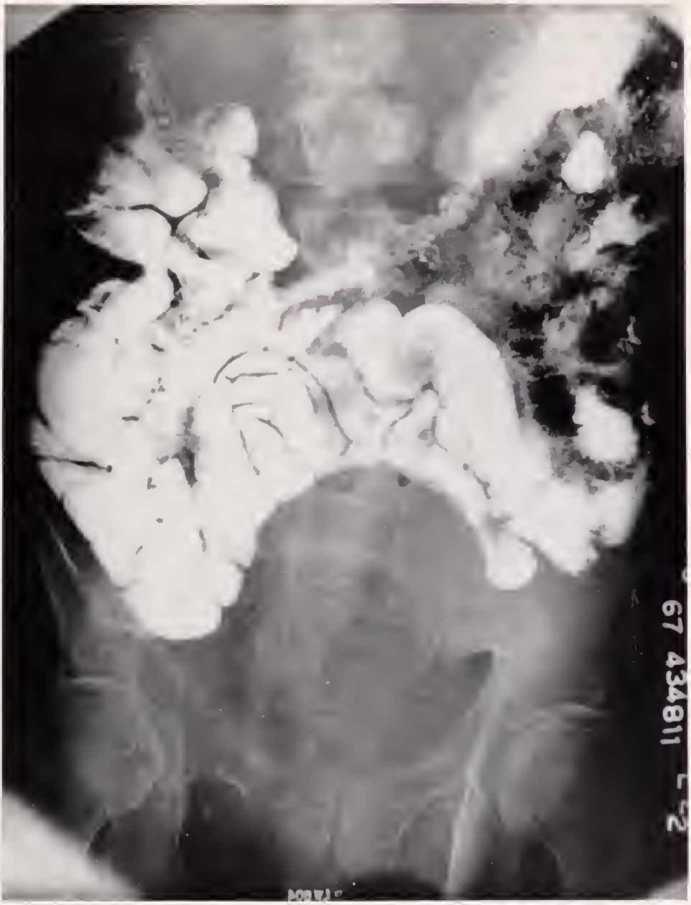

FIG. 1. Upper gastrointestinal series, 2½ hours after swallowing barium. There is gastric retention, slight small bowel dilatation, and distention of the urinary bladder.

in the right ulnar and left peroneal nerves were abnormal. An electromyogram showed severe neuropathic abnormality. The electroencephalogram was normal. Bone marrow aspiration showed erythroid hyperplasia (plasma cells 4.5%). Twenty-five microcuries of Cr^{51} albumin were injected intravenously and a 96 hour stool collection contained 0.9% of the administered dose of Cr^{51}, indicating no significant gastrointestinal protein loss. Sigmoidoscopy was normal to 12 cm. Esophageal motility studies showed an absence of motor activity in the distal 7/8 of the esophagus. There was amyloid deposition in the vascular walls in the rectum, which was birefringent under polarized light with the congo red stain. There was amyloid in the blood vessel walls, lamina propria, and muscularis mucosa but the villous pattern of the small bowel was normal. There was amyloid in the blood vessel walls in the submucosa and the muscularis of the stomach. The skeletal muscle biopsy showed atrophy typical of a neurogenic pattern.

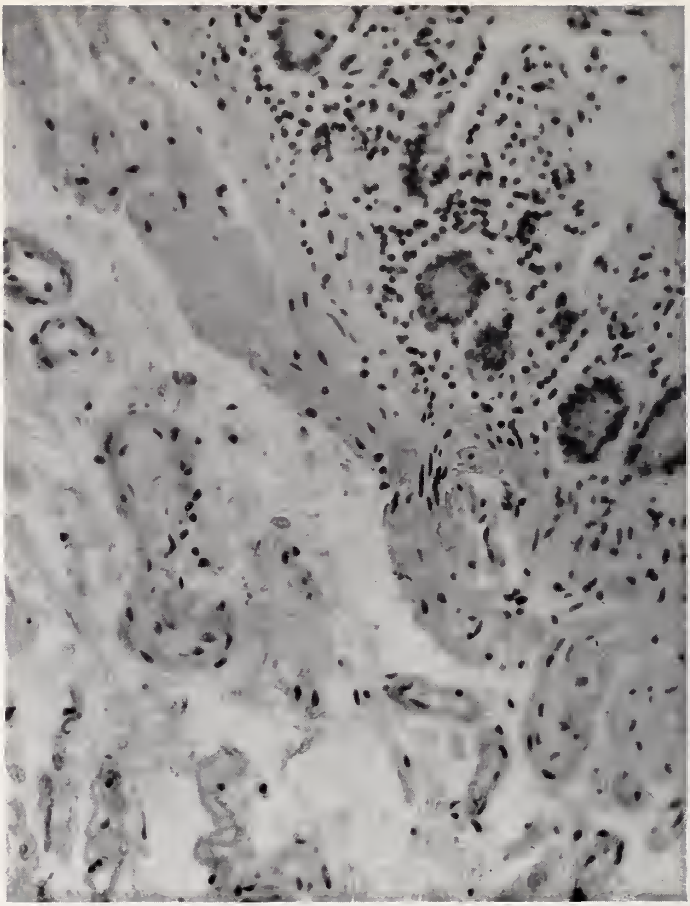

FIG. 2. Photomicrograph of small bowel with congo red stain × 150. Amyloid is seen in the walls of the submucosal vessels.

On admission the patient was maintained on intravenous fluids until the dehydration was corrected. A gluten free diet and medium chain triglycerides were then started but he continued to have 4 to 8 loose stools per day. On September 29, 1967 he spiked a temperature of 102°F, and urine, stool, and blood cultures grew Aerobacter aerogenes which responded promptly to kanamycin. In October 1967 he was given 10 cc of gamma globulin per day intramuscularly for a 3 week period, followed by 22 units of fresh frozen plasma over a 4 week period. There was no improvement in his condition during this period, although there were no further episodes of fever. The hospital course was characterized by progressive malaise and inanition, and he expired on December 13, 1967.

At autopsy there was amyloid in the muscularis and walls of the submucosal vessels in the esophagus, stomach, small bowel (Fig. 2), and colon. There were interstitial deposits in the endocardium, myocardium, and the blood vessels of the heart. There were amyloid deposits in the connective

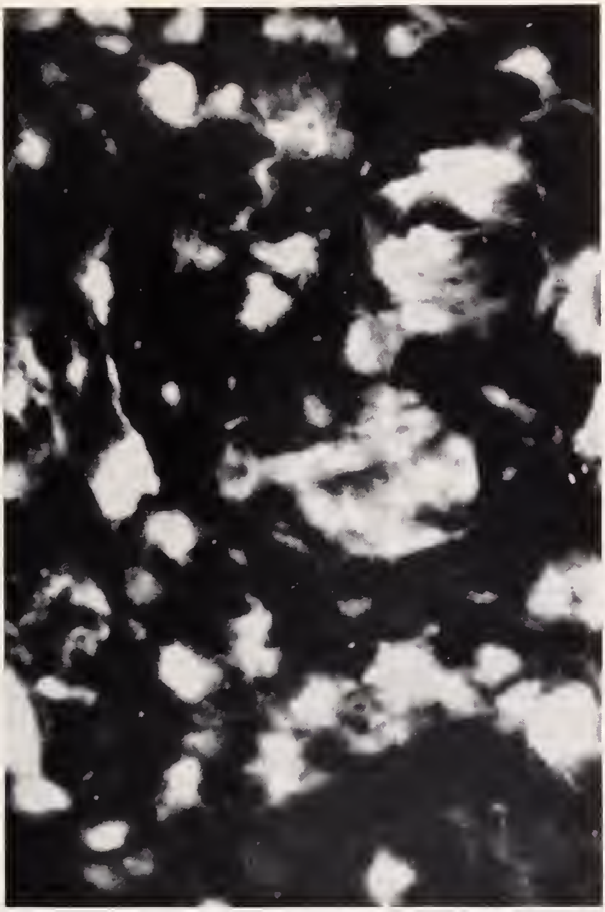

FIG. 3. Photomicrograph of autonomic nerve ganglia with thioflavin T stain × 560. Amyloid deposits exhibit fluorescence.

tissue and blood vessels of the liver, kidneys, spleen, adrenals, thyroid, and parathyroid. There was no amyloid deposition in the glandular stroma of the pancreas. There was amyloid infiltration of the autonomic nerve ganglia (Fig. 3).

Discussion

This patient presented clinically with evidence of a chronic disease characterized by weight loss and malaise, followed by steatorrhea. The initial differential diagnosis included pancreatic insufficiency, diabetic steatorrhea, bacterial overgrowth of the bowel, celiac disease, and amyloidosis. The low xylose absorption, serum carotene, and elevated fecal fat excretion documented the malabsorption. In pancreatic insufficiency, the xylose absorption is normal and there is a response to treatment with pancreatic extract. In diabetic steatorrhea, the intravenous glucose tolerance test is abnormal. In bacterial overgrowth, treatment with appropriate antibiotics produces clinical improvement.

In classic adult celiac disease, treatment with a gluten free diet produces a remission. The radiologic appearance of the small bowel, which is usually helpful in diagnosing malabsorption syndromes, was nondiagnostic. The persistent symptoms of peripheral and autonomic neuropathies were compatible with the syndrome of primary amyloidosis, but biopsy of the small bowel was necessary to establish the diagnosis.

The gastrointestinal tract is involved histologically in 30 percent of cases of amyloidosis examined at autopsy (2), but it does not commonly produce the initial symptoms. There is no correlation between the amount of amyloid deposition in the gastrointestinal tract and the degree of symptoms (3–5). Neurologic deficits occur in about 14 percent of cases of primary amyloidosis (6–10). The combination of steatorrhea, peripheral, and autonomic neuropathies in primary amyloidosis has rarely been described in the literature and usually the diagnosis was not made until autopsy (11, 12).

The pathophysiology of the malabsorption in primary amyloidosis is not well understood. In this case the intestinal villi were normal; therefore, the uptake of nutrients by the membrane of the mucosal cell should not have been impaired. There was no amyloid deposition in the mucosal cell, so the transfer of substances from the luminal to serosal surface of the cell should not have been mechanically blocked. There was no amyloid infiltration of the basement membrane of the mucosal cell, so the release of substances into the body fluids should have been normal. There was a small amount of amyloid deposition in the lamina propria, which could explain only a slight impairment of uptake into body fluids. Amyloid deposition in the submucosal vessels would not cause malabsorption.

Disturbances of gastrointestinal motility were seen clinically in decreased to absent esophageal motility, barium retention in the stomach on the upper gastrointestinal series, and decreased transit time through the small bowel. A small polyethylene tube, which was passed for the purpose of studying intestinal lipid digestion, did not advance beyond the pylorus 48 hours after it had entered the body of the stomach. Histologically, there was amyloid infiltrating the submucosal and myenteric ganglia, and the circular and longitudinal muscle layers of the small bowel. This may have interfered with segmental contractions in the bowel, with resultant impairment of mixing of the ingested food stuffs. Inadequate mixing of food with digestive enzymes, such as pancreatic lipase and bile salts, may prevent the hydrolysis of triglycerides and the formation of micelles. Fields and Duthie have shown that the intestinal pH after vagotomy was higher with a decreased amount of bile salts, potentially leading to an impairment of digestion (13). The disturbance of the gastrointestinal tract in primary amyloidosis appears to be one of maldigestion rather than malabsorption. However, this does not explain why the xylose absorption was decreased, and the administration of medium chain triglycerides, which are absorbed in the absence of bile salts and pancreatic enzymes, were ineffective.

Histologically, there was also amyloid deposition surrounding the thoracolumbar autonomic ganglia. This deposition may have interfered with the sympathetic innervation of the small bowel, which may have led to the diarrhea. This has been demonstrated experimentally in dogs by Verney and Vogt, who found intractable diarrhea in dogs after sectioning the prevertebral ganglia (14).

Unexplained chronic diarrhea in the diabetic, which is usually found in association with peripheral or autonomic neuropathy, is similar to that seen in amyloidosis. The signs of the autonomic neuropathy such as impotence, pupillary abnormalities, impaired bladder emptying, and orthostatic hypotension are seen in both diabetes and amyloidosis. The diabetic diarrhea occurs predominately at night and there may be spontaneous remissions, but in amyloid diarrhea there is no diurnal pattern and the course is always progressive. When steatorrhea is present in diabetes, all measures of fat absorption show impairment, but generalized malabsorption, as of vitamin B_{12} and xylose, is not present (15). The autonomic ganglia are usually histologically normal in diabetes, but in amyloidosis there is deposition of congo red staining material. In diabetes the small bowel biopsy is normal, and in amyloidosis there is deposition in the bowel wall.

Further studies are necessary to document the maldigestion in amyloidosis, such as measuring the products of digestion at different levels in the small bowel following the instillation of a known amount of fat through an intestinal tube, and especially the distribution of intraluminal lipid into micellular and oil phases.

Immunoglobulin levels in amyloid steatorrhea have not previously been reported. It is known that decreased immunoglobulins may occur in primary amyloidosis (16). The mechanism of diminished immunoglobulins in this patient are not known. There was no excessive urinary or gastrointestinal loss of these molecules, as might have been expected. In normal individuals, IgA containing plasma cells predominate in the lamina propria of the small bowel, and may be the only immune globulin in the normal feces. The decreased serum immunoglobulins, especially IgA as seen in this patient, have recently been reported to be associated with malabsorption. This patient had no past history of frequent respiratory infections, as is seen in primary hypogammaglobulinemia (17). There was no lymphoid hyperplasia or giardia lamblia in the stools, as reported by Hermans et al (18). This patient did not respond to a gluten free diet, as the case of IgA deficiency and steatorrhea reported by Crabbe and Hermans (19). Conn and Quintiliani (20) reported improvement in a patient with hypogammaglobulinemia and diarrhea after intramuscular injections of gamma globulin, and Binder and Reynolds (21) reported improvement in a patient with secondary hypogammaglobulinemia and diarrhea after administration of fresh frozen plasma. Neither gamma globulin nor fresh frozen plasma decreased the diarrhea in our patient.

Summary

A case of primary amyloidosis presented clinically as a malabsorption syndrome associated with peripheral and autonomic neuropathies. The presence of amyloid was documented by stomach, small bowel, and rectal biopsies and

confirmed at autopsy. The pathophysiology of malabsorption in primary amyloidosis is difficult to explain in anatomical terms. Disturbances in intestinal motor activity related to involvement of autonomic ganglia may play a role in the syndrome. The reduction of immunoglobulins of all classes is of interest.

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The Hemodynamic Effects of Diphenylhydantoin in Patients with Fixed Rate Pacemakers*

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Introduction

Diphenylhydantoin (DPH) is now widely used as an antiarrhythmic agent, but there have been reports of a negative inotropic effect on the heart of the experimental animal (1–4). In the three previous studies of the hemodynamic effects of DPH in humans, one showed no hemodynamic changes (5), another a depressant effect on the right ventricle (6), and the third a decrease in left ventricular function (7). This study was undertaken to determine the hemodynamic effects of DPH in patients with heart block and permanently implanted pacemakers, because such patients provide a means of studying drug effect at rest and exercise independent of changes in heart rate.

Method

Nine patients with complete heart block and permanently implanted fixed rate pacemakers were studied. There was complete ventricular capture by the pacemaker at rates of 68 to 83 beats/minute. Five of the patients were males and four were females, and their ages ranged from 47 to 80 years (Table I). Seven patients had arteriosclerotic heart disease, one had rheumatic aortic insufficiency, and one had hypertensive heart disease. Although six of the patients required treatment for congestive heart failure at some time in the past, none showed clinical evidence of congestive failure, and none were receiving cardiac medications at the time of the study. All studies were performed in the basal, postabsorptive state, without premedication. With the patient in the supine position, an 18T Cournand needle was placed in the brachial artery for measurement of the systemic arterial pressure and for the withdrawal of blood for the dye-dilution curves. A vinyl catheter was inserted percutaneously into an antecubital vein and advanced into the right atrium or right ventricle under pressure monitoring for the injection of dye. Cardiac outputs were performed by the indicator-dilution technique, utilizing indocyanine green, a cuvette densitometer, and a constant speed withdrawal pump. Pressures were measured with ultra-low displacement unbonded strain gauges. All data were recorded on a multichannel oscilloscopic photographic recorder. Cardiac outputs, mean brachial artery pressures, and

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TABLE I

Patient	Age	Sex	Diagnosis	Pacing Duration	CHF* in Past	Cardio-megaly	Implantation Site
T.P.	56	F	ASHD*	8 Wk	+	+	TV*
R.V.	75	F	ASHD	4 Yr	+	+	E*
J.F.	73	M	ASHD	2 Yr	+	+	TV
J.P.	82	M	ASHD	8 Wk	+	+	E
S.L.	85	M	HCVD*	8 Da	0	+	TV
L.W.	47	M	RHD*	3 Mo	+	+	TV
P.I.	76	M	ASHD	2 Mo	0	+	TV
M.L.	66	F	ASHD	2 Yr	0	+	TV
A.T.	80	F	ASHD	2 Wk	+	+	TV

* CHF = congestive heart failure; ASHD = arteriosclerotic heart disease; HCVD = hypertensive heart disease; RHD = rheumatic heart disease; TV = transvenous; E = epicardial.

atrial rates were determined at rest and for 15 minutes following injection of DPH (4 mg/kg to a maximum of 250 mg) into the right atrium or ventricle at a rate of 50 mg/minute. In five patients a constant level of exercise was performed on a bicycle ergometer, with the rate and work load constant during all exercise periods. Maximum tolerance was determined before drug administration. Exercise studies were repeated 15 minutes following DPH injection.

Results

Cardiac index (CI) was determined in eight patients. In one (J.P.), CI fell from 1.82 to 1.52 $l/min/m^2$ immediately after DPH. In the remaining patients, CI did not change appreciably from control levels. Average CI (based on six observations, see Table II) revealed no significant deviation from rest values.

Mean brachial artery pressure was measured in eight patients, and a fall in pressure was seen in seven patients immediately after DPH injection. The reduction was greater than 10% in four patients (S.L., R.V., M.L., and A.T.). The average reduction in mean brachial artery pressure, based on six observations, was 14 mm Hg (106 to 92 mm Hg or 11%, see Table III). Maximum fall was 23 mm Hg.

Atrial rates (AR) were measured in eight patients. Reduction occurred in all eight, and a greater than 10% reduction was seen in four (S.L., R.V., M.L., and A.T.). Because AR was calculated only when P waves were readily recognizable, recordings were not possible at each time period for all patients, and averages are not valid. The maximum fall in atrial rate was 32 beats/minute.

No atrial arrhythmias were produced, and in no instance was there failure of pacemaker stimulus to capture.

Hemodynamic measurements were made during exercise in several patients before, and 15 minutes after, diphenylhydantoin administration (CI in five,

TABLE II

Cardiac Index ($L/min/m^2$)

Patient	Before DPH	After DPH Immed	After DPH 5 min	After DPH 15 min	Exercise Before DPH	Exercise After DPH
L.W.	2.29	2.17	2.18	2.49	3.12	3.51
S.L.	1.78	1.86	1.94	1.97	3.21	3.27
T.P.	1.78	1.65	1.72	1.98	3.33	3.15
R.V.	1.37	1.40	—	1.43	1.90	1.90
P.I.	2.05	2.01	1.93	1.90	2.46	2.25
M.L.	2.30	2.05	2.17	2.16	—	—
J.F.	2.78	2.57	2.94	2.56	—	—
J.P.	1.82	1.52	—	1.74	—	—
Average*	2.16	2.05	2.15	2.18	2.80	2.82

* Based on six observations only.

TABLE III

Mean Brachial Artery Pressure (*mm Hg*)

Patient	Before DPH	After DPH Immed	After DPH 5 Min	After DPH 15 min	Exercise Before DPH	Exercise After DPH
L.W.	116	111	113	120	143	136
S.L.	83	70	78	79	96	92
T.P.	116	106	110	110	—	—
R.V.	125	102	—	108	—	—
P.I.	106	109	110	—	138	131
M.L.	126	106	110	112	—	—
J.P.	78	72	85	85	—	—
A.T.	114	88	104	96	—	—
Average*	106	92	100	100	126	119

* Based on six observations only.

mean brachial artery pressure in four, AR in three). In all patients so studied, there was no difference between pre- and post-drug exercise parameters.

All patients tolerated the procedure without incident.

Discussion

Conn, Kennedy, and Blackmon (5) have shown that 250 mg of DPH injected intravenously over 3 to 5 minutes caused no significant change in cardiac output, systemic or pulmonary artery pressures, heart rate, peripheral resistance, or stroke volume. Leiberson et al (7), on the other hand, found transiently depressed left ventricular function in nine patients with heart disease following the same dose of DPH. In their study, left ventricular end-diastolic pressure rose in all patients (above normal limits in four), and left ventricular dp/dt fell in eight. Mean cardiac index did not change, but an examination of individual changes in CI over 30 minutes after injection showed significant increases in two patients (27 and 38%), and decreases in five patients (17 to 25%). A recording of hemodynamic effects was begun 5 minutes after DPH. Studies in animals, however, have shown that negative inotropic effects are maximum within 40 seconds of injection, and may be dissipated within 5 minutes (2). Mierzwiak et al (6) assessed right ventricular function in four patients after DPH, and found decreased right ventricular dp/dt in two patients, and increased right ventricular end-diastolic pressure in another patient. Incidentally, one patient developed a marked nodal bradycardia following drug administration.

In none of the studies cited above was the heart rate controlled by artificial pacing or were exercise measurements performed. This study indicates that, although DPH in physiologic doses causes transient reduction in systemic arterial pressure and atrial rate, this depression was not sufficient to affect cardiac output at a constant ventricular rate.

Summary

The cardiovascular effects of diphenylhydantoin were studied in nine patients with complete heart block and permanently implanted fixed rate pacemakers. After administration of 4 mg/kg (maximum 250 mg) of DPH into the right atrium or ventricle, cardiac index did not change significantly from control values over 15 minutes of observation. There was an immediate reduction in mean brachial artery pressure (average 106 to 92 mm Hg, maximum 23 mm Hg), and atrial rate (91 to 79 beats/minute, maximum 32 beats/minute). There was no significant effect on exercise hemodynamics 15 minutes following drug administration.

It is concluded that DPH causes a transient decrease in systemic arterial pressure and atrial rate, but no significant change in cardiac index at a fixed ventricular rate.

Acknowledgment

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The Neurological Complications of Ewing's Sarcoma: Incidence of Neurologic Involvement and Value of Radiotherapy*

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Introduction

Ewing's sarcoma is a primary malignant tumor of bone, first recognized by Lücke in 1866, and subsequently described by Hildebrandt, Markwald, and others (3). James Ewing (6, 7) characterized it most extensively, and sought to distinguish it from other entities, such as lymphoma and reticulum cell sarcoma. He named it diffuse endothelioma of bone and, after osteogenic sarcoma, it is the most common primary malignant bone tumor (10, 12), with a predilection for children and adolescents. It begins in the shaft more frequently than in the end of the long bones, and rapidly metastasizes to other bones, lungs, and skull (3). The tumor is relatively radiosensitive, but because of its rapid dissemination, it has a very unfavorable prognosis, with only 5 percent surviving 5 years (10). Although it involves the nervous system in over 15 percent of cases (4), there is a paucity of discussion of the neurological complications of Ewing's tumors in the literature. We are presenting 32 patients with neurological involvement in a series of 100 cases of Ewing's sarcoma. Attention is drawn to the radioresponsiveness of the nervous system metastases.

Method

From 1950 to 1965, 100 patients with Ewing's sarcoma were admitted to the Sloan-Kettering Memorial Hospital Cancer Center for diagnosis and treatment. In a retrospective study, 32 patients were found to have neurological involvement, and they constitute the basis for this report. All patients were seen in consultation by a staff neurologist, neurosurgeon, or a resident in neurology. In the last 3 years many of the cases were seen by one of the two authors, and recently neuroradiological procedures, such as angiography, pneumoencephalography, or myelography have been performed.

Results

Ewing's sarcoma is primarily a tumor of childhood, and our series reflects this; 24 cases with neurological complications of Ewing's sarcoma were between the ages of 4 and 16 years. Twenty-two patients were male and 10 patients were female. Table I shows the frequency of the neurological symptoms of which the patients complained. Headaches were a common complaint, occurring in 17 cases, and were usually associated with increased intracranial

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TABLE I

Frequency of Neurological Symptoms in 32 Cases

Symptoms	No. of Cases
Headaches	17
Neck and/or back pain	17
Double vision	10
Paresthesiae	8
Nausea and vomiting	7
Blurred vision	5
Disturbance of urinary function	5
Dizziness	4
Auditory disorder	2
Visual hallucinations	2

pressure. Neck and/or back pain occurred as frequently as headaches, and were felt to herald local bony metastasis with root compression. Diplopia was found in 10 patients, and was a prominent feature of patients with elevated intracranial pressure. Seven of these patients complained of nausea and vomiting. Auditory disorder and visual hallucinations occurred in 2 patients.

Tables II and III are tabulations of the positive findings on neurological examination. It is apparent from these data that the major abnormalities involved the peripheral roots, nerves, and cerebrum and, to a lesser extent, brain stem and cranial nerves. Twenty-five patients had generalized muscle weakness, a finding which is difficult to evaluate since the majority of patients were malnourished and had received neurotoxic drugs. Of the 32 patients examined, 19 had pain on straight leg raising, and 15 patients had increased intracranial pressure manifested by papilledema. Vertebral column tenderness was found in 12 patients. Nystagmus, painful neck flexion, Babinski signs, and hemiparesis occurred with about equal frequency. Horner's syndrome and proptosis were seen in about 6 cases, and 4 patients had generalized seizures. Distended scalp veins was a rare finding (2 cases).

In the examination of the cranial nerves, the optic nerve was found to be directly affected in 4 cases. The oculomotor nerve was affected in 2 cases, and the jugular foramen group was occasionally affected (the hypoglossal nerve was affected in 3 cases). Two-thirds of patients with papilledema had abducens palsies.

Table IV summarizes the anatomic diagnoses made on the basis of the clinical examination. Patients with Ewing's tumors had dysfunction of the cerebrum (12 cases), or brain stem dysfunction (14 cases) at some stage in the progression of the disease. Radiculopathy (26 cases), particularly lumbosacral root disease (17 cases), and neuropathy (12 cases) were also prominent findings.

Table V summarizes the laboratory results. In the examination of the spinal fluid, 19 patients had a pressure greater than 220 millimeters of water, while pleocytosis and increased total protein were found in about one-quarter of all

TABLE II

Frequency of Neurologic Signs in 32 Cases

Signs	No. of Cases
Muscle weakness	25
Pain on straight leg raising	19
Vertebral column percussion tenderness	12
Paraparesis	10
Nystagmus	9
Pathological reflexes	9
Pain on neck flexion	9
Hemiparesis	8
left	7
right	1
Horner's syndrome	6
Proptosis	5
Seizures	4
Organic mental syndrome	3
Visual field defect	3
Aphasia	2
Enlarged, distended scalp veins	2
Deafness	2

TABLE III

Frequency of Cranial Nerve Abnormalities in 32 Cases

Cranial Nerves	No. of Cases
II—papilledema	15
blindness	4
VI	10
XII	3
III	2
IX, X, XI	1
VII	1
VIII	2

patients (9 cases). The electroencephalogram was bilaterally abnormal in 7 of the cases, and showed a unilateral focal abnormality in 4 patients. Angiography by either the brachial or carotid routes demonstrated a cerebral mass lesion in 6 cases, and obstructive disease of the cerebral venous sinuses in 2 of the patients. Myelography showed an extradural block in 4 cases. It should be noted here, however, that many of the cases of neurologic involvement seen prior to 1960 did not have neuroradiologic studies, and these figures might be considerably higher had all patients been investigated.

Postmortem examinations were obtained in 13 patients, 6 of whom demonstrated extradural tumor in the cranial meninges, and 3 in the spinal meninges. The 1 case demonstrating a parenchymal cerebral metastasis is described in Case 5. Two cases showed tumor infiltrating the wall of the superior sagittal

TABLE IV

Frequency of Presumed Anatomic Diagnoses in 32 Cases

Anatomic Diagnosis	No. of Cases
Encephalopathy	12
Brain stem dysfunction	14
Cranial neuropathy	5
Retro-orbital tumor	5
Myelopathy—cervical	1
thoracic	5
Radiculopathy—cervical	9
lumbosacral	17
Peripheral neuropathy	12

TABLE V

Laboratory Results

Procedure	Result	No. of Cases
Spinal fluid (32 cases)	Elevated pressure >220 mm H_2O	19
	Elevated protein >50 mg%	9
	Increased cells >4	8
Electroencephalography (13 cases)	Bilateral slowing	7
	Focal abnormality	4
	Electrical seizure	1
	Normal	1
Angiography (9 cases)	Mass lesion	6
	Venous sinus occlusion	2
	Normal	1
Pneumoencephalography (4 cases)	Ventricular asymmetry	1
	Normal	3
Myelography (5 cases)	Extradural block	4
	Normal	1
Postmortem (13 cases)	Extradural tumor	9
	brain	6
	spinal cord	3
	Parenchymal tumor (brain)	1
	Venous sinus occlusion	1
	Normal	2

sinus (Figs. 9 and 10), and 2 patients had normal brains at autopsy. Figure 1 demonstrates the typical appearance of Ewing's sarcoma in the cranial meninges, with sheets of small oval or spindle-shaped cells with prominent nuclei and sparse cytoplasm.

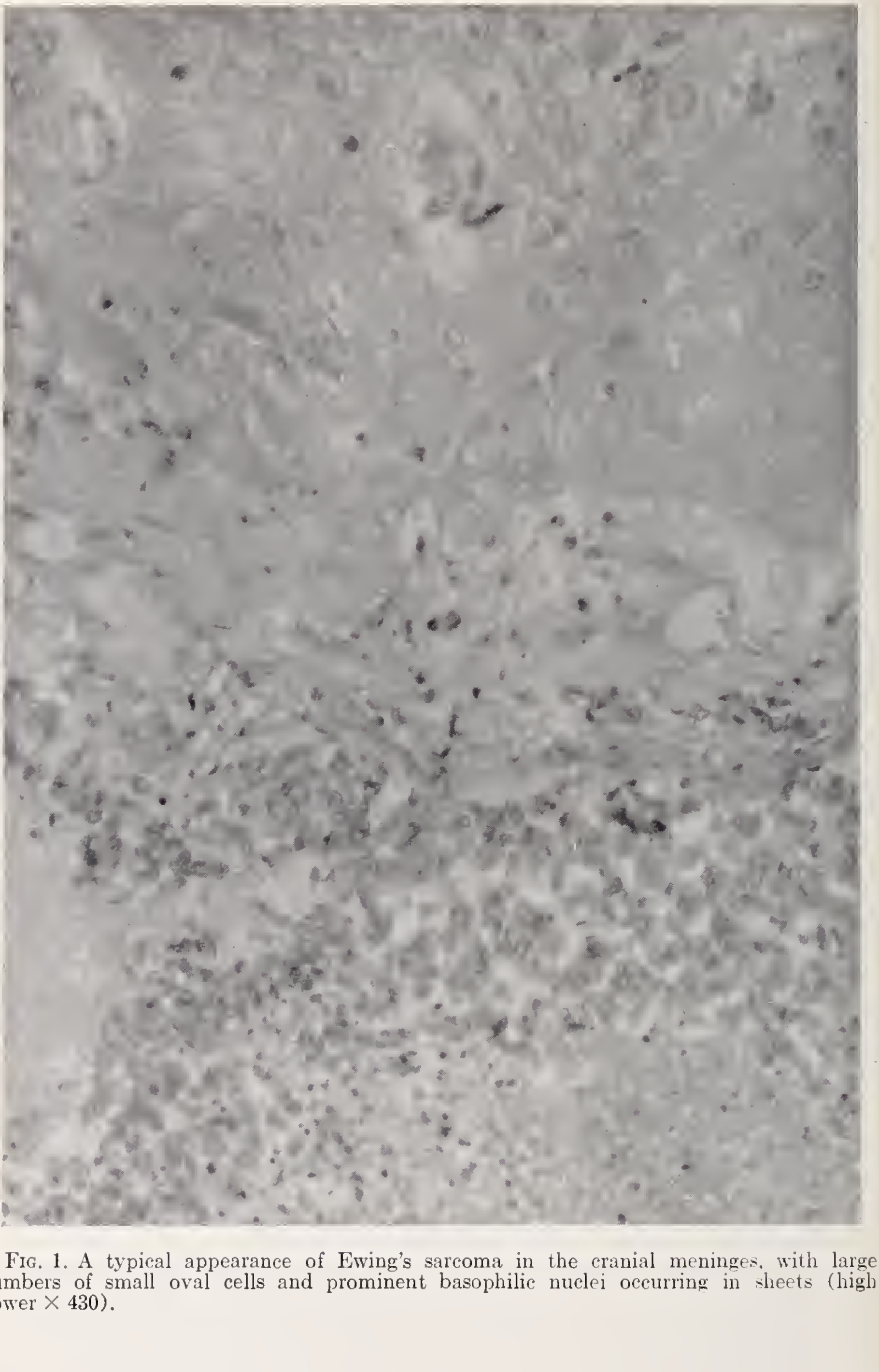

FIG. 1. A typical appearance of Ewing's sarcoma in the cranial meninges, with large numbers of small oval cells and prominent basophilic nuclei occurring in sheets (high power × 430).

Case Reports

The following eight case reports have been selected to illustrate the various neurological syndromes manifested by Ewing's tumors, and also to document the diagnostic procedures and emphasize the therapeutic value of radiation therapy.

Case 1. (R.Y., 244533). In February 1963 a 12-year-old white boy was found to have an Ewing's tumor of the right ulna, and underwent a right midshaft amputation. In May 1964 the patient began to limp on the left leg, and had pain in the low back and suprapubic regions. He also became incontinent of urine, numbness in the feet and toes developed, and he had hyperesthesiae in the rectal area. Neurological examination at that time revealed an intellectually dull boy who exhibited defects in orientation, memory, and calculation. Cranial nerve examination revealed diminished wrinkling of the left obicularis oculi, slightly indistinct left optic disc margin, and poor adduction of the right eye. Motor examination showed paraparesis and poor sphincter tone. Ankle jerks were absent. Sensory examination revealed defective temperature perception over the L_5 to S_4 dermatomes, and hyperesthesia to pinprick over the S_2 to S_4 segments. Vibration and position sense were normal.

A myelogram revealed a complete extradural obstruction at L_4 (Fig. 2). The total protein of the cerebrospinal fluid was 59 mgm%. The patient received 2500 r to the lumbosacral area. A repeat myelogram 1 month later showed decrease of the block, with defects noted in the L_4-L_5 region (Fig. 3). The patient had definite improvement in bladder function and in motor power in his legs.

In October 1964 the patient had recurrent pains in his legs, flank, and abdomen, and became unable to walk. A bony lesion at T_{12} was noted on x-ray, and he received 2000 r to that area. At this time neurological examination demonstrated dull intellect and papilledema in the left eye, definite weakness of the left upper extremity, paraparesis, and a left Babinski sign. A lumbar puncture was normal. The electroencephalogram was bilaterally abnormal with suppression of brain activity on the left, and slow wave activity on the right (Fig. 4). In this case the mass lesion was demonstrated to be on the side of low voltage, suppressed activity, rather than on the side of high voltage, slow wave activity, as demonstrated on the EEG. A right carotid angiogram was performed, and the anterior cerebral artery and internal cerebral veins were found to have shifted from left to right, indicating a left cerebral mass (Fig. 5). The patient received 2250 r to the T_{10}-S_3 area and 2100 r to the whole skull, with improvement of the weakness in the left upper extremity. At this time the patient is still alive but confined to a bed and chair existence.

Comment: The patient had proved multiple areas of central nervous system involvement from metastatic Ewing's sarcoma. The role of radiation therapy in this case is clearly established by the two myelograms, which showed disappearance of the extradural block.

Intracranial mass lesion, as shown by angiography, is rare in Ewing's sarcoma, and as far as we know has not been previously reported in the literature.

Case 2. (F.R., 244331). An 8-year-old white girl was diagnosed as having Ewing's sarcoma of the left humerus in January 1963. In April 1963 she noted blurring of vision in the left eye, headache, vomiting, and diplopia. Examination revealed amaurosis of the left eye. The left pupil was larger than the right and reacted sluggishly to light. There was a right abducens palsy. The jaw deviated to the right. Lumbar puncture was normal, as was an x-ray of the skull. The patient received radiotherapy to the left optic nerve and chemotherapy, without noticeable effect.

In May 1963 the patient complained of bone pain and right leg pain. Examination revealed the previously noted cranial nerve abnormalities. In addition, paraparesis was noted. Myelography revealed a complete extradural obstruction at T_4 (Fig. 6). Following 1868 r, the T_4 block disappeared, and the patient regained the use of her lower extremities (Fig. 7).

The patient suffered from systemic metastases, and died in November 1963. Autopsy permission was refused.

Comment: Again, the value of radiation therapy is well shown with the pre-radiation and post-radiation therapy myelograms. During the period of myelographic improvement, the clinical status of the patient also improved.

Case 3. (*R.A., 259689*). A 10-year-old white boy was found to have Ewing's sarcoma of the left humerus in May 1962. In May 1963 he complained of diplopia. Examination revealed bilateral papilledema, bilateral abducens weakness, and deviation of the jaw to the right. There was evidence of right motor Vth nerve, left VIIth nerve, left VIIIth nerve, and right XIIth nerve involvement. There was dysdiadochokinesis in the left upper extremity, and moderate weakness of the right lower extremity. Sensory examination revealed hypesthesia to pinprick over the anteromedial surface of the right thigh and medial right calf. Reflexes were equal and normal, and there were no pathological reflexes. Lumbar puncture revealed an opening pressure in excess of 600 mm of water, with normal protein. Electroencephalogram showed bilateral cerebral dysfunction with bursts of 3–4 cps, up to 70 mv, right more than left. A right brachial angiogram revealed two large epidural masses in the parasagittal and midline regions, compressing the superior sinus (Fig. 8). Pneumoencephalography did not show evidence of a mass lesion. The patient received radio-

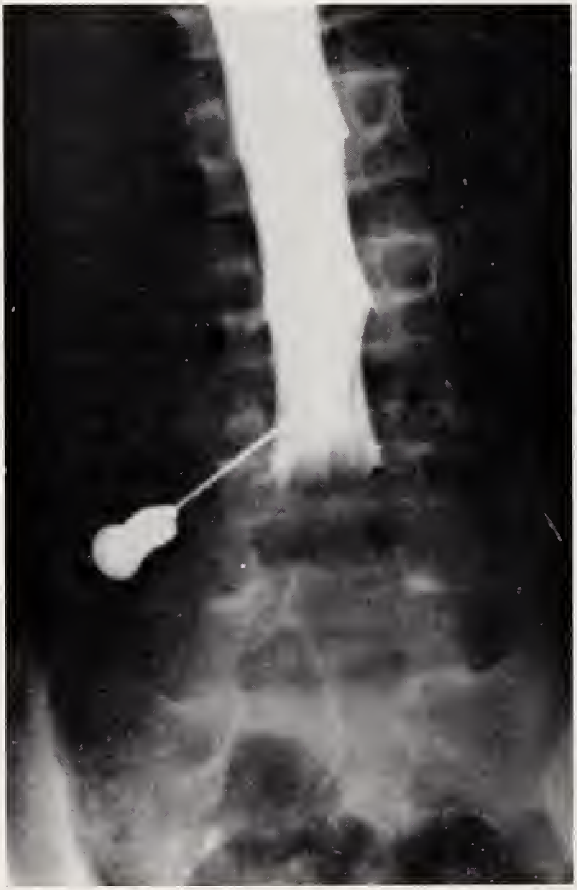

Fig. 2. Case 1. Lumbar myelogram. There is a complete extradural type of block to the pantopaque column at L_4.

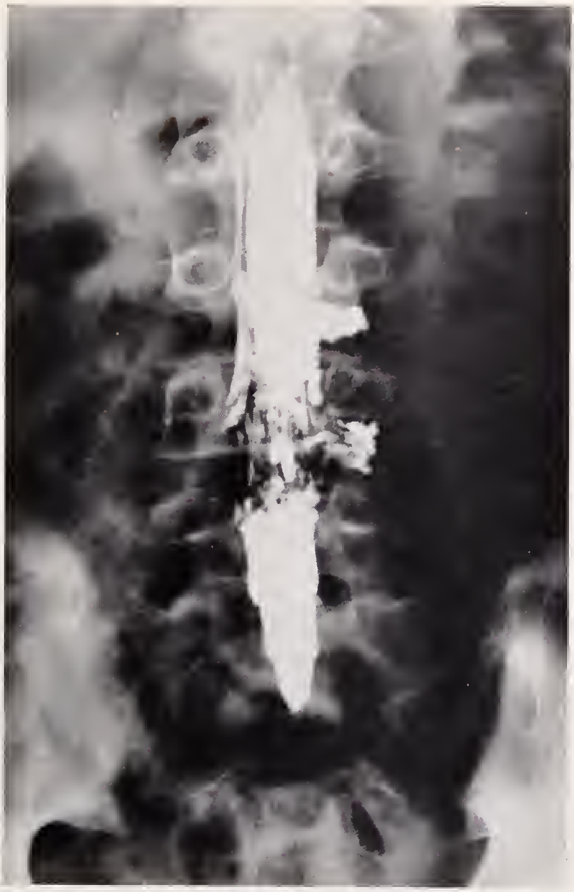

Fig. 3. Case 1. Lumbar myelogram. The previously noted complete block of radiopaque material has now become a partial block following radiotherapy.

therapy, and the tumor masses on the skull decreased in size. The papilledema and increased cerebrospinal fluid pressure regressed. A right brachial angiogram was repeated, and the epidural masses were definitely shown to be smaller (Fig. 9). During the next 11 months the patient became totally blind and suffered from severe motor neuropathy secondary to Vincristine. Proptosis of the right eye was noted before death in June 1964.

Postmortem examination revealed extensive infiltration in the dura by tumor, with a large 5 x 4 x 2 cm mass in the left parietal region, indenting the surface of the brain, and a similar mass in the right parietal region. The superior sagittal sinus was infiltrated and almost occluded by tumor (Fig. 10). Sections of the brain revealed focal necrotic areas of 3–4 mm in the cortex of both cerebral hemispheres, especially in the right temporal lobe. The optic nerves and tracts were atrophic.

Comment: This was a complex case, with multiple cranial lesions and epidural tumor involving the superior sagittal sinus. It is possible that sinus occlusive disease, secondary to tumor, was the cause of the increase in cerebrospinal fluid pressure. The hypothesis is supported by the clinical improvement which occurred after the epidural masses were shown by angiography to be decreased in size following radiotherapy.

Case 4 (R.A., 253322). A 6-year-old white girl developed Ewing's sarcoma of the right radius in April 1961. She was seen first by the Neurology Service in February 1963 for diplopia, headache, vomiting, and pain in the neck. Examination revealed bilateral papilledema with hemorrhages, exudates, and a tendency for the eyes to converge. Lumbar puncture revealed a pressure of 570 mm of water, with a normal protein. A left carotid angiogram revealed good filling of the arterial system, but no visualization of the torcula, transverse sinus, or posterior portion of the superior sagittal sinus. A pneumoencephalogram was normal. The patient was noted to have prominent superficial veins over the forehead and temporal regions. One day later the cerebrospinal fluid pressure was over 600 mm of water, and the protein was 194 mgm%. Chemotherapy and radiotherapy were started, and a spinal tap 17 days later revealed a cerebrospinal fluid pressure of 240 mm of water, and a protein of 54 mgm%. Reexamination demonstrated improvement of the papilledema and diminution of the scalp vein distention. Diplopia continued, however. The patient's systemic disease continued to progress, and she died in July 1963. Permission for postmortem examination was refused.

Comment: The increased cerebrospinal fluid pressure was definitely established. In view of the normal pneumoencephalogram, venous sinus disease is a likely cause of the increased cerebrospinal fluid pressure. This case is included as a demonstration of significant improvement in signs of increased intracranial pressure, although the patient's course was not significantly improved.

Case 5. (M.M., 236105). This patient was found to have Ewing's sarcoma of the right femur in May 1960. In November 1960 she had a left temporal scalp mass, which responded

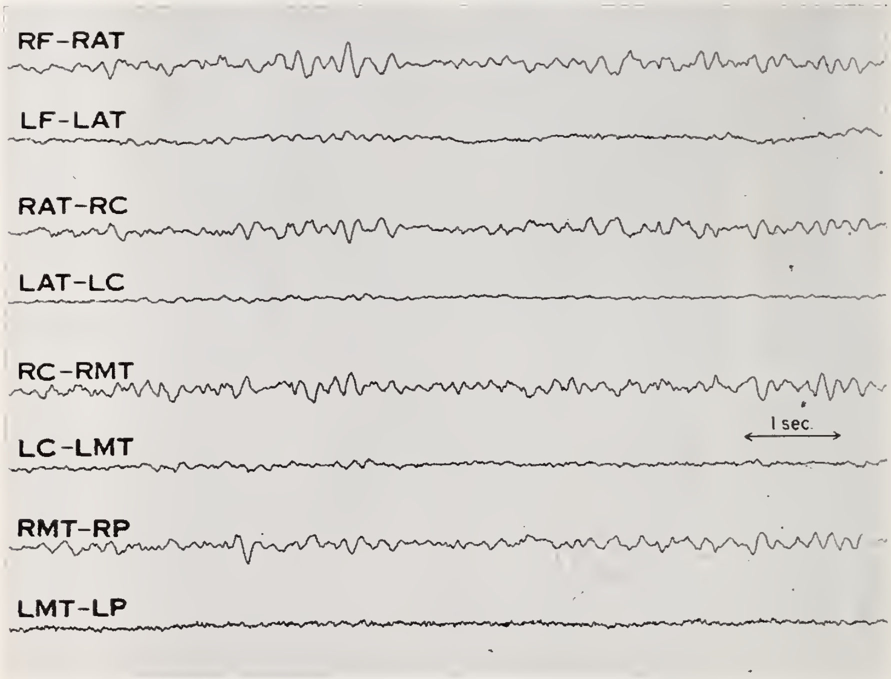

Fig. 4. Case 1. Electroencephalogram. There is marked suppression of brain activity during hyperventilation in all left sided electrodes, with slow wave bursts accentuated in the right temporal electrodes. RF = right frontal; RAT = right anterior temporal; LF = left frontal; LAT = left anterior temporal; RAT = right anterior temporal; RC = right central; LC = left central; RMT = right midtemporal; LMT = left midtemporal; RP = right parietal; LP = left parietal.

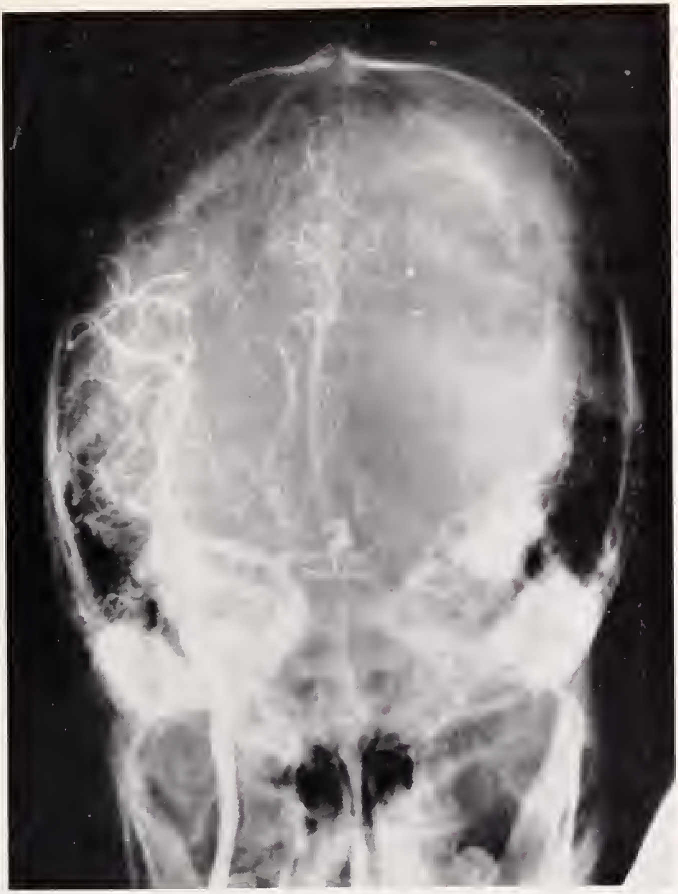

FIG. 5. Case 1. Right carotid angiogram. There is a shift of both anterior cerebral arteries to the side of injection, indicating a left cerebral mass lesion.

to radiation. In September 1961 the patient was seen at another hospital with headaches, seizures, and papilledema. Lumbar puncture revealed a cerebrospinal fluid pressure of 280 mm of water, with 50 mgm% protein. An electroencephalogram was bilaterally abnormal, with right temporal focal accentuation. Bilateral carotid angiograms demonstrated a right parietal extradural mass, and a questionable one in the left temporal region. The right parietal mass was removed by craniotomy. Following the operation, the papilledema receded and there were no further seizures. She was transferred to Memorial Hospital in October 1961. At that time she was found to have an organic mental syndrome, papilledema, left homonymous hemianopsia, left hemiparesis, and a left hemisensory defect. The patient also made aphasic errors. She died in April 1964 because of systemic disease.

Postmortem examination revealed a large extradural tumor mass, compressing the left temporal lobe. There were multiple small extradural masses, and there was an extensive area of encephalomalacia of the right parietal and occipital lobes under the craniotomy defect. In addition, 3 cm posterior to the right frontal pole, and 1.5 cm from the midline there was a cortical and subcortical partially hemorrhagic parenchymal tumor metastasis. It proved to be Ewing's sarcoma on microscopic examination.

Comment: Intramedullary brain metastasis, proved by postmortem examination, is an extremely rare finding in Ewing's sarcoma.

Case 6. (*P.K., 254802*). An 11-year-old white boy developed Ewing's sarcoma of the left femur in October 1961. In April 1964 he was noted to have a right frontoparietal scalp mass. In July 1964 the patient experienced headaches, vomiting, diplopia, and hazy vision in the right eye. Examination revealed the patient to be lethargic and to have an organic mental syndrome. He had diminished visual acuity in the right eye, bilateral papilledema, left third nerve palsy, and left facial weakness. He dragged his left leg, had a left hemiparesis, left Babinski sign, and left hemisensory defect. Lumbar puncture revealed an opening pressure of over 600 mm of water, and proteins of 103 mgm% and 84 mgm%. Electroencephalogram revealed a 3–6 cps, 50 mv right temporal focus and 3–4 cps, 150–200 mv *left* frontal focus, with shifting slow wave foci bilaterally (Fig. 11). Right brachial angiogram revealed filling only of the vertebral-basilar system. Bilateral carotid angiography demonstrated a right frontal mass, with massive deviation of the anterior cerebral artery to the *left* (Fig. 12). The patient lapsed into coma, and was started on radiotherapy and intravenous urea. Remarkable improvement was noted after radiotherapy (Fig. 13), as exemplified by the striking change in the EEG, as well as in the clinical examination. The patient became alert, but still had bilateral blindness and mild hemiparesis.

Comment: Bilateral papilledema in this case was secondary to a huge intracranial mass. It is not clear if the amaurosis was secondary to optic nerve pressure by tumor, or was related to general increase in intracranial pressure. Of interest is the marked improvement following radiotherapy. Despite the huge size of the extradural mass, a remission was obtained following radiotherapy.

Case 7. (*R.A., 236168*). A 14-year-old boy was diagnosed as having Ewing's sarcoma of the right femur in January 1961. In March 1962 he had a flaccid paraplegia and sensory level at T_7. There was aphasia and right facial weakness. Bilateral carotid angiography re-

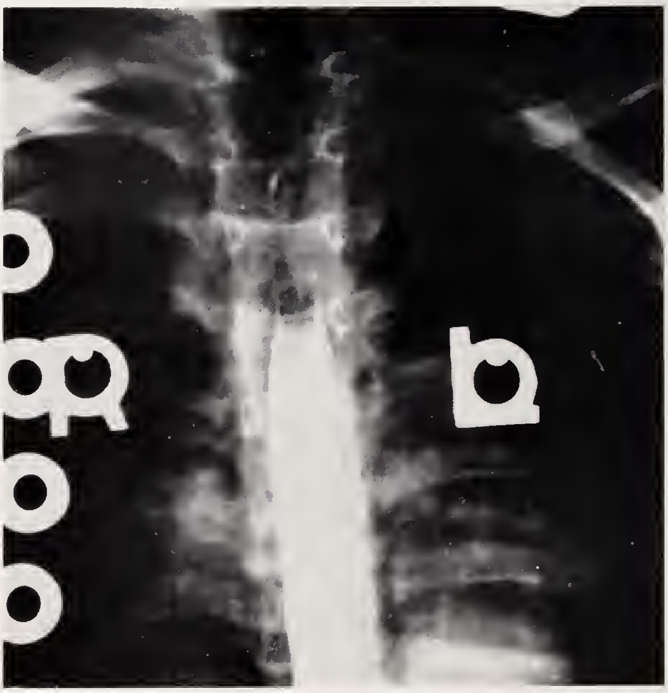

FIG. 6. Case 2. Cervical myelogram. There is a complete extradural type of block of the pantopaque column at the T_4 vertebral level.

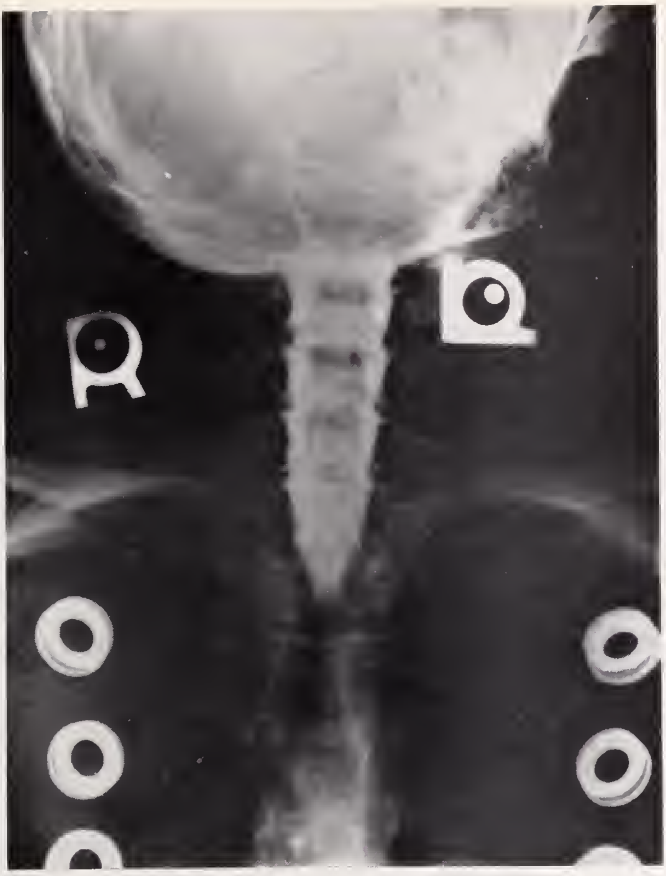

FIG. 7. Case 2. Cervical myelogram. The previously demonstrated block of the radiopaque column at T_4 has now disappeared following radiotherapy.

vealed elevation of the left middle cerebral artery and shift of the left anterior cerebral artery to the right, indicating a left anterior temporal mass. The patient died in March 1962, before myelography could be performed.

Permission for postmortem examination was not obtained.

Case 8. (S.K., 210849). A 7-year-old white boy was found to have Ewing's sarcoma of the left femur. In December 1962 the patient had pain in the right neck, headache, and diplopia. Examination revealed bilateral papilledema and vertical diplopia on left lateral gaze. Lumbar puncture showed an opening pressure of 460 mm, but was otherwise normal. Electroencephalogram was bilaterally abnormal, right more than left, with bursts of 2–3 cps, up to 150 mv, and asymmetry with diminished alpha on the left. Pneumoencephalography was normal.

One month later the patient was found to have right IXth, Xth, XIth, and XIIth cranial nerve paresis with weakness, atrophy, and fasciculations of the right side of the tongue. Repeat pneumoencephalography was again normal. Radiotherapy was given to the posterior fossa and occipital bone, with complete regression of all neurologic abnormalities.

In April 1964 he was found to have a bruit over the right occipital bone, with pain in that area. The previously described cranial nerve palsies recurred. Lumbar puncture revealed an initial pressure of 240 mm of water and 12 cells, 72% monos, and 28% polymorphs.

Total protein was 68 mgm%. Electroencephalography showed bursts and runs of 2.5–4.0 cps, up to 120 mv activity in the occipital and posterior temporal regions, with higher voltage on the right. Skull x-rays revealed multiple lytic lesions, predominantly in the right parietal regions. The child received chlorambucil and radiotherapy, but in October 1964 was noted to have a left abducens weakness, complete right facial weakness, diminished hearing on the right, and complete paralysis of the tongue with dysphagia and dysarthria. Further progression occurred, with pulmonary and other bony metastases. The patient died. A postmortem was not obtained.

Comment: Occlusion of the jugular vein by tumor which also involved the IXth, Xth, XIth, and XIIth cranial nerves on the right side, and subsequent sagittal sinus occlusion, is

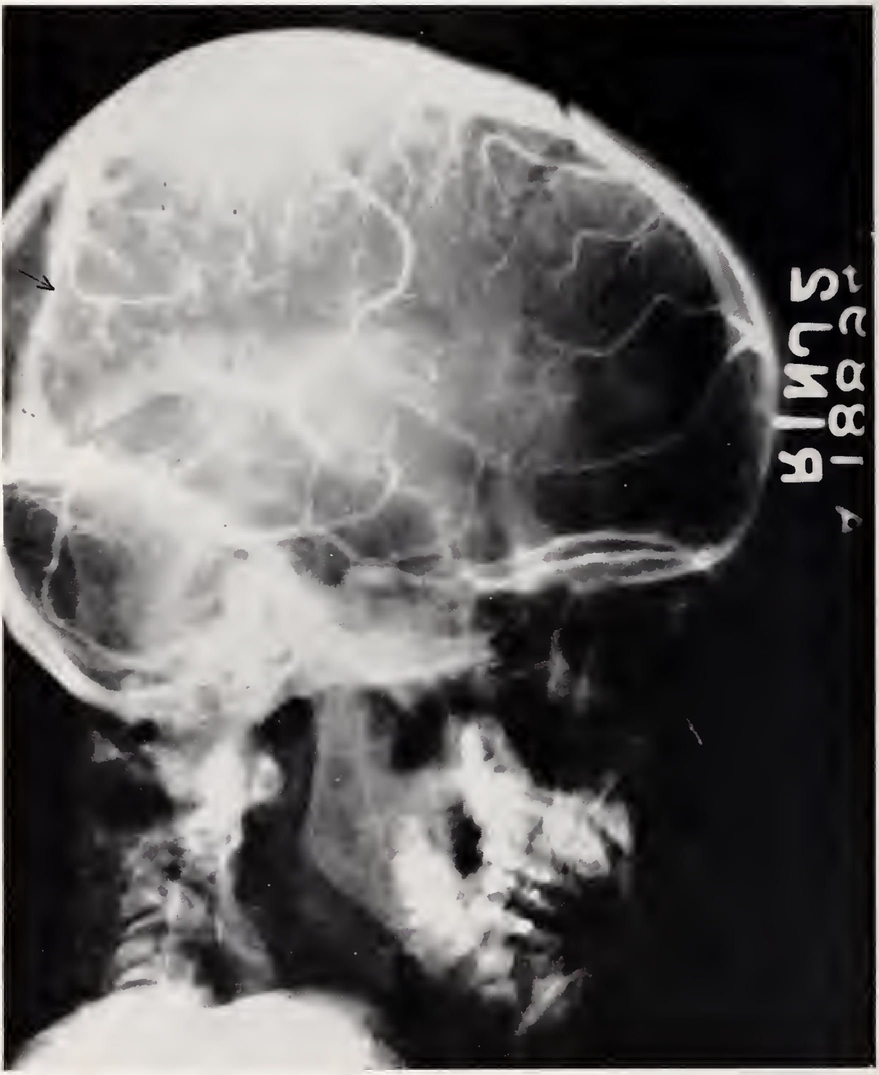

Fig. 8. Case 3. Right brachial angiogram. Lateral view. In the late venous phase the superior sagittal sinus and torcula region are markedly distorted inwardly away from the inner table of the calvarium by a large avascular extradural mass.

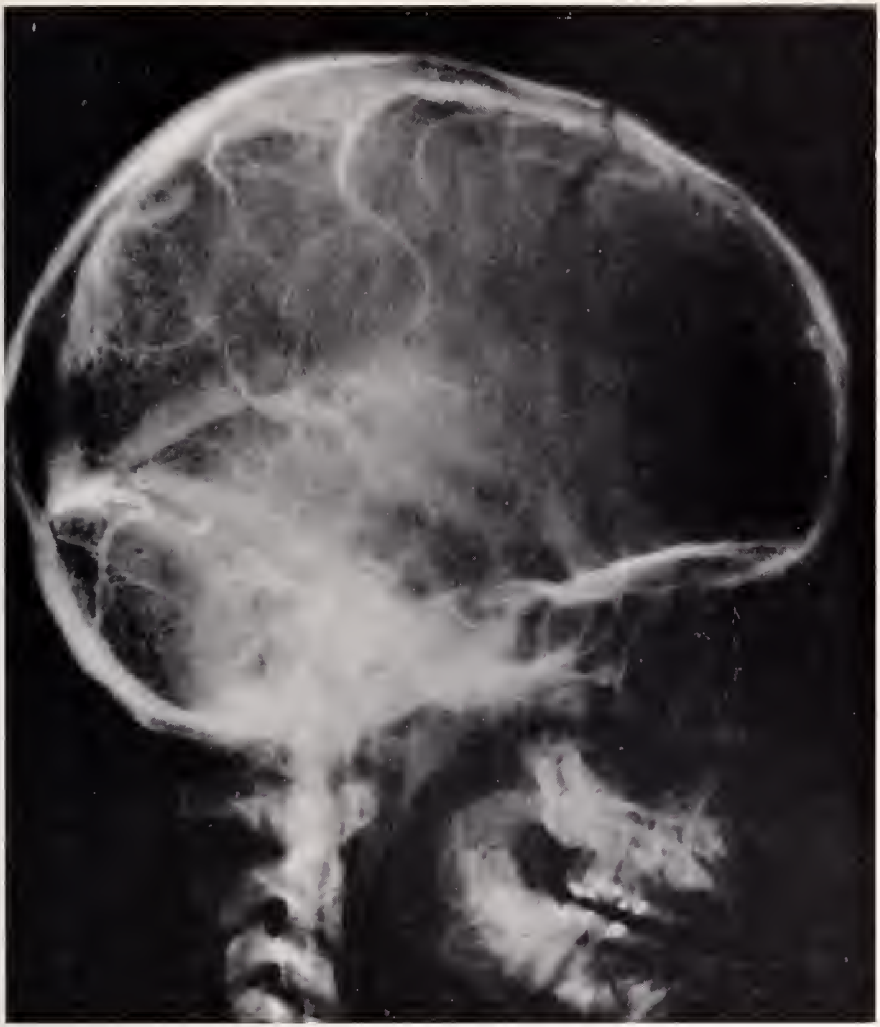

FIG. 9. Case 3. Right brachial angiogram. Lateral view. Compared to Figure 8, the compression of the superior sagittal sinus and torcula is considerably diminished following radiotherapy.

a postulated mechanism for the production of the increased intracranial pressure. The small to normal-sized ventricles in the face of the sustained elevated cerebrospinal fluid pressure (600 mm) was construed as supportive evidence for this hypothesis.

Discussion

James Ewing (7) in 1940 mentioned skull metastases, but there was no discussion of neurological symptoms. In 1948, Coley, Higinbotham, and Bowden (4) reported from Memorial Hospital that 16% of the patients had a neurological lesion. Two patients with sacral involvement had lumbosacral root symptoms, urinary retention, and lower abdominal or leg pain. Eight patients had paraplegia from involvement of the thoracic spine. There was one case

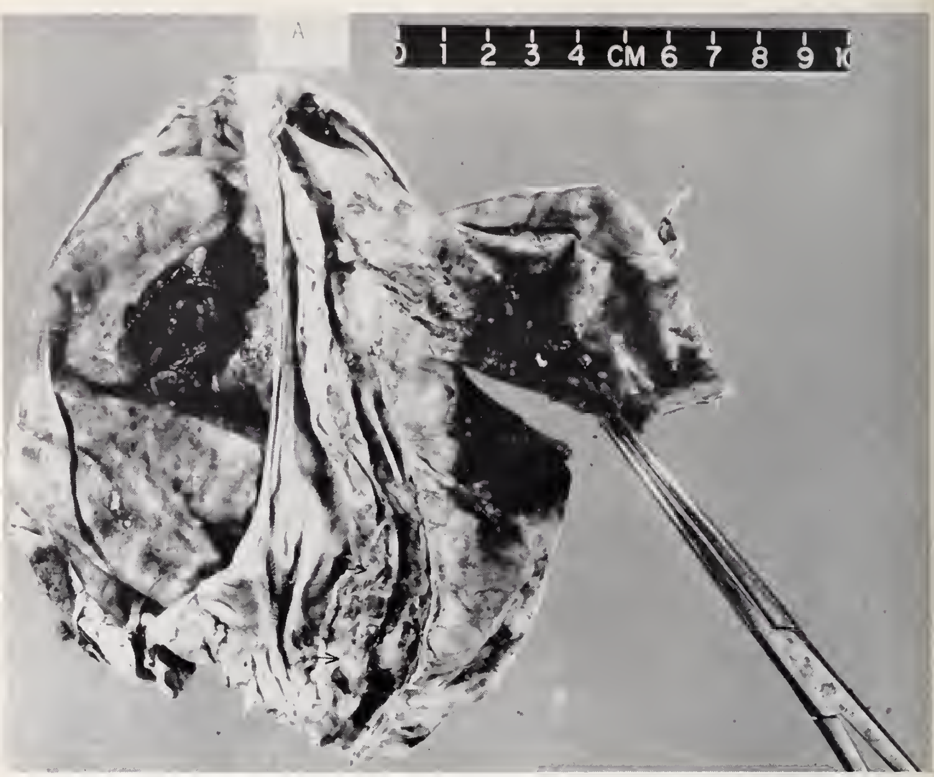

Fig. 10. Case 3. Postmortem specimen. The superior sagittal sinus and torcula region are heavily infiltrated with tumor. There are hemorrhagic areas in the dura over the convexity, due to pressure from extradural tumor.

each of blindness, ophthalmoplegia, quadriplegia, and bilateral proptosis. Two cases of VIIth nerve paresis were mentioned. Skull and vertebral metastases were the second and third most common clinical sites of involvement after pulmonary spread. At autopsy it was found that the skull and spine were frequently involved.

In the case records of the Massachusetts General Hospital (13), a 19-year-old boy with perineal dysesthesiae, urinary retention, and paraparesis, with normal cerebrospinal fluid and myelogram, was found to have Ewing's sarcoma of the sacrum. Fontaine, Darry, Warter, and Stoll (8) described a case of lumbar root pain treated conservatively for 5 years as a herniated disc. The patient, in whom Ewing's sarcoma was found, developed leg paresthesiae, weakness of the flexors of the right leg, absent ankle jerk, and hyperesthesiae over the S-1 distribution, with point tenderness of L_5-S_1. Myelography demonstrating an extradural Ewing's sarcoma with spinal cord compression was described by Köves (11), and Carteri and Constantini (2) reported a case with a frontal mass and a normal carotid angiogram. Other authors (1, 5, 9, 17) also described spinal cord involvement from extradural tumors at vari-

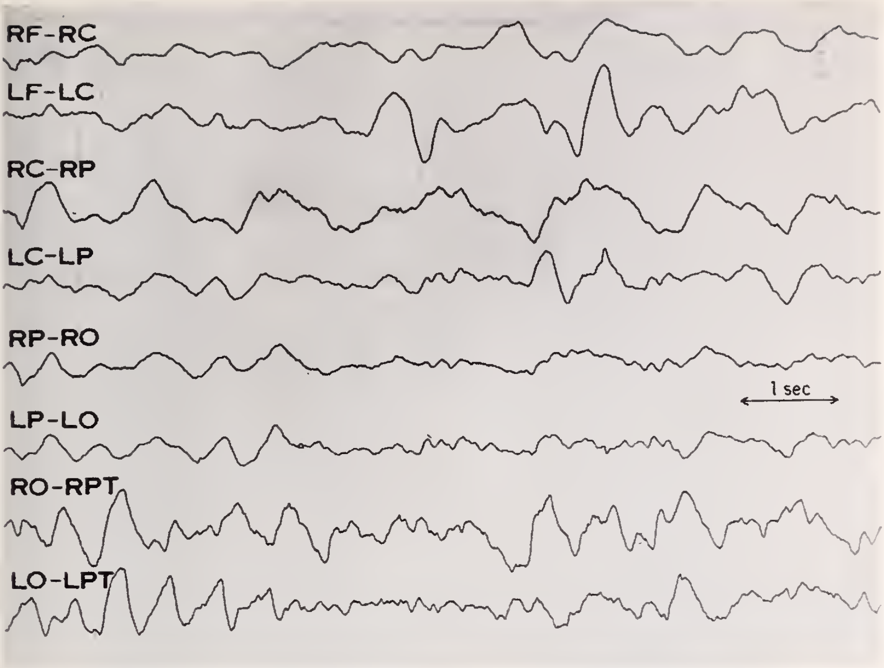

FIG. 11. Case 6. Electroencephalogram. There is marked bilateral slow wave activity down to .75 cps in the right frontal and left temporal regions. RF = right frontal; RC = right central; LF = left frontal; LC = left central; RP = right parietal; LP = left parietal; RO = right occipital; LO = left occipital; RPT = right posterior temporal; LPT = left posterior temporal.

ous levels. There have, however, been no reports with intramedullary cerebral metastases, intracranial venous sinus obstruction, or space-occupying extradural cerebral masses demonstrated by angiography in the literature, nor has there been recorded a systematic description of the frequency of the various neurological signs and symptoms. Finally, there have been only two cases recorded in which neuroradiological procedures were used to demonstrate the tumor (2, 11), and only one was abnormal (11).

It can be readily seen from our data that neurological involvement from Ewing's sacroma is a common occurrence (32/100). Usually, the cranial nerves are involved concomitantly with skull metastases, and it is presumed that the nerves are involved at the base of the skull where they are probably pressed upon by bony metastases. Although many patients have proved skull metastasis at this time, it is not uncommon to have cranial nerve dysfunction in Ewing's sarcoma with no obvious skull x-ray abnormalities. As was noted, the most common cranial nerves involved were the IInd, IIIrd, XIIth, and jugular foramen group, in that order.

Papilledema and increased intracranial pressure were seen in one-half of the cases, and are the most common neurological manifestations of Ewing's tumors. The cause of increased intracranial pressure is not clear in all cases.

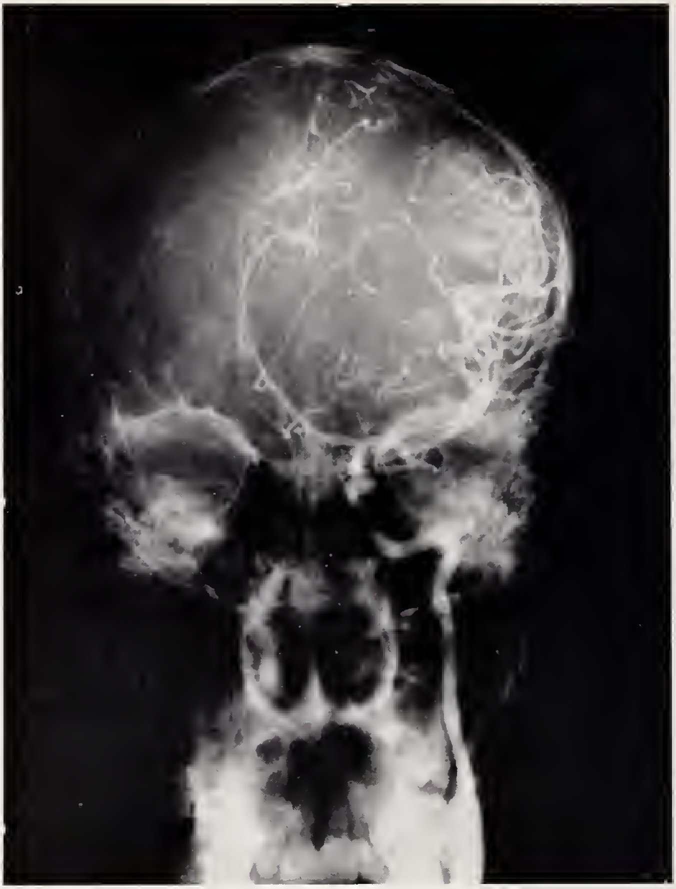

FIG. 12. Case 6. Right carotid angiogram. Anterior-posterior view. There is a huge shift of the anterior cerebral artery to the left, indicating a right frontal mass.

Large intracranial masses, either in the supratentorial or infratentorial regions, are one possible explanation of the increased intracranial pressure. This was shown in six of the cases. Involvement of sagittal sinus and torcular region with venous occlusive disease was proved in two patients, Cases 3 and 4. The diagnosis of sagittal sinus torcular compression with venous obstruction and papilledema can be made antemortem by angiography, as is shown clearly in Case 3.

Cerebral manifestations of Ewing's sarcoma are common, and at some stage in the disease may occur in more than 90 percent of the cases. The literature states that intramedullary metastases from Ewing's sarcoma are rare. In our cases there was only one patient with proved intramedullary Ewing's sarcoma of the brain (Case 5). Six cases had definite intracranial mass lesions as shown by angiogram, but it was not clear in two cases whether they were intramedullary, or extradural. These cases demonstrated that cerebral angiography is important in defining the site of cerebral pathology.

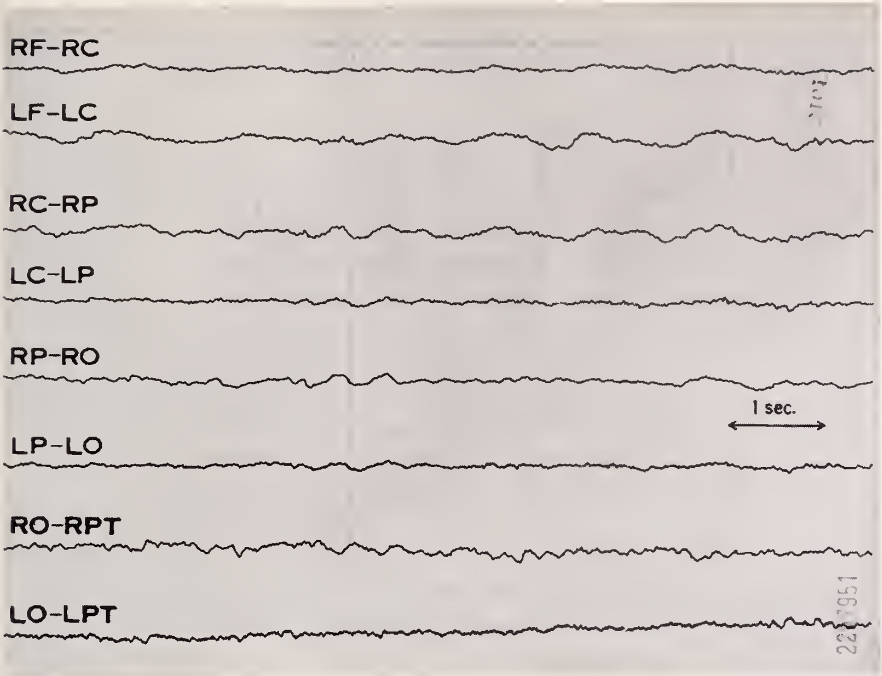

Fig. 13. Case 6. Electroencephalogram. Compared to Figure 11 there is marked improvement in the EEG following radiotherapy, although the record continues to be bilaterally abnormal. RF = right frontal; RC = right central; LF = left frontal; LC = left central; RP = right parietal; LP = left parietal; RO = right occipital; LO = left occipital; RPT = right posterior temporal; LPT = left posterior temporal.

One of the most common neurologic manifestations of Ewing's tumor is radiculopathy in the lumbosacral area, less in other regions. Myelograms have shown extradural mass lesions pressing upon these roots. Spinal cord compression has been seen in six cases, and five myelograms have been performed, four of which were abnormal. These have shown extradural compression defects. The importance of myelography should be stressed, as the tumor is very radiosensitive, and excellent results are possible with radiotherapy when extradural compression defects are demonstrated. Cases 1 and 2 emphasize this fact, as a repeat myelogram performed after chemotherapy and radiotherapy showed the block to have disappeared. These patients made excellent recovery from spinal cord dysfunction, as seen frequently in this series, but died later from systemic spread of the Ewing's tumor.

The treatment of the neurological manifestations of Ewing's sarcoma is of great importance. It can be seen from these cases that almost all patients showed some radiosensitivity of their tumors. Most patients with neurological manifestations had definite improvement from radiotherapy. This was true in all areas of the nervous system including spinal cord, roots, cranial nerves, and cerebrum. This fact should be stressed, since frequently patients with systemic manifestations of metastatic disease are not treated when neurological symptoms appear. We cannot over-emphasize the importance of radiotherapy for treatment of the neurological manifestations. In our series of cases, the neurological lesions receded with treatment, and the cause of death was usually due to systemic metastases.

In view of our experience, surgery appears to have no place in the treatment of neurological manifestations of Ewing's sarcoma, although laminectomies and craniotomies are frequently considered. While our case numbers are not great, experience with radiosensitive tumors, such as Ewing's sarcoma, leads us to believe that medical treatment with radiotherapy is the most effective treatment in complete myelographic blocks with spinal cord compression. In spite of the radiosensitivity of the tumor, the long term survival of Ewing's sarcoma is poor. Less than 5 to 10% survive 5 years. In general, the patients die from systemic illness, and not from neurological complications which are improved by radiotherapy.

Summary

Ewing's sarcoma is the second most common primary malignant bone tumor, and has a predilection for children and adolescents. Although the tumor is radiosensitive, because of the rapidity of its dissemination, only 5% survive 5 years.

In a retrospective study of 100 cases of Ewing's tumors, 32 patients were found to have neurologic involvement, and one-half of these were studied by neuroradiologic procedures.

All areas of the nervous system were affected. Elevated intracranial pressure with papilledema occurred in over 50% of the cases. Dysfunction of the peripheral roots occurred most frequently, with peripheral nerve, brain stem, and cerebral involvement occurring less often. The IInd and IIIrd cranial nerves, as well as the jugular foramen group, were involved by bony metastases in 10 to 20% of the cases.

Laboratory results commonly revealed elevated cerebrospinal fluid pressure, pleocytosis, and elevated total protein. Electroencephalography showed diffuse and focal abnormalities. Angiography demonstrated extradural cerebral mass lesions and sagittal sinus obstruction, while myelography showed extradural blocks. Postmortem examinations confirmed these lesions, and there was one case of parenchymal cerebral metastasis, a very rare occurrence.

Eight clinical cases are presented with neuroradiologic documentation. These cases demonstrate the radioresponsiveness of cerebral and spinal extradural masses, as well as occlusive sagittal sinus disease.

A review of the literature is presented and no cases with abnormal angiograms, sagittal sinus occlusions, or parenchymal cerebral metastases were found.

Our experience indicates that Ewing's tumors of the nervous system are radiosensitive, and that vigorous diagnostic work-up and medical therapy are indicated.

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A Comparison of the Syndromes Associated with a Partial Deletion of the Short Arms of Chromosomes #4 and #5*

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Introduction

The cri du chat syndrome is characterized clinically by a peculiar cry resembling the mewing of a cat in the first week of life; severe mental and physical retardation; failure to thrive; microcephaly; a rounded face with simple, low set ears; hypertelorism; oblique downward slanted (antimongoloid) palpebral fissures; epicanthus; a broad nasal bridge; simian lines; and abnormal dermatoglyphics (1–3). Other less constant findings are strabismus; abnormal ear canals; minor skeletal abnormalities; hypospadias; cortical atrophy; congenital heart defects; short neck; high arched palate; and micrognathia (4). The cri du chat syndrome was first described by Hirschhorn and Cooper in 1961 (5), then by Lejeune et al in 1963 (1), and soon afterwards by several other authors (6–13), so that now approximately 40 children have been reported in the literature having this syndrome. This clinical picture is associated with a partial deletion of the short arm of one of the B group chromosomes (chromosomes #4 and #5) (14).

The B group chromosomes are not easily distinguished by size or length of the arms. In fact, if one were to measure the long arms of the four B chromosomes in one cell and predict from the relative length of the deleted chromosome whether it is a #4 or #5 chromosome, that is, whether it is a late or an early replicator, one would be wrong about one-third of the time (15). There is, however, an association between replication pattern and length of the long arm in the B group chromosomes (16). On the assumption that the length differences represent actual differences in the amount of genetic material, Miller et al conclude that chromosome #4 and chromosome #5 differ in both length and replication pattern. The pair which terminates replication of its long arms late in the synthetic period has been called chromosome #4, and the shorter pair, whose long arms replicate earlier, has been called chromosome #5 (17, 18). The variability of replication pattern is so small that in using this criterion for classification of replication pattern, only approximately 1% of the chromosomes would be misclassified (15).

Several authors have published cases also with a partial deletion of the short arm of one of the B group chromosomes, but in which the clinical picture has been reported to differ from that of the cri du chat syndrome. Autoradiography revealed that the abnormal chromosomes belonged to the B group chromosome with the late DNA synthesis in its long arms. Hirschhorn's patient died before autoradiography could be performed, and Sidbury also did not do autoradiographic analysis (19–26).

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The clinical picture of partial monosomy of chromosome ※4 is as follows: Severe mental and physical retardation; failure to thrive; hypertelorism; low birth weight; simple, low set ears; microcephaly, and micrognathia. Beaked nose; preauricular sinus; cleft lip, jaw, and palate; sacral sinus; cryptorchism; and hypertrophy of the clitoris have been reported in some cases of partial deletion of the short arm of chromosome ※4, and infrequently in cases of cri du chat syndrome. The following group of signs have not been mentioned in any case of cri du chat syndrome: pupillary ectopy; coloboma of the iris; exopthalmus; dislocation of the hip; hypospadias; epileptic manifestations; dysplasia of skin ridges; and the presence of volar pads. Van Kempen and Jongbloet stress that the dysplasia of the dermal ridges and presence of volar pads may be of considerable importance in differential diagnosis (25). In addition, there is a high preponderance of females having cri du chat syndrome, whereas both sexes appear to be equally affected in partial monosomy of chromosome ※4 (4).

Van Kempen and Jongbloet (25), and Leao et al (23) emphasize that these are two clinically distinct syndromes. However, if they are clinically distinct, one should be able to put a new patient into one category or the other. This is sometimes possible. If a newborn has a cat-like cry, one can be reasonably certain that it has a deletion of the 5th chromosome. If a newborn has a beaked nose, with midline defects, and no characteristic cry, one can predict that it has partial monosomy of chromosome ※4. However, if the patient is not newborn, but is several years old, and has lost the cry, there is a very large percentage of misdiagnosis.

Methods

The hypothesis that the clinical distinction is real was tested by screening 2,000 male and female patients at an institution for the mentally retarded, specifically looking for patients with the presumed characteristic clinical features associated with the partial deletion of the short arm of chromosome ※4. The features used in selecting the cases to be studied were as follows: very low I.Q.; flat nasal bridge; small beaked nose; hypertelorism; and micrognathia. These features are not specific, and Van Kempen and Jongbloet (25) state that they are present in many different general chromosomal abnormalities. Each feature by itself is meaningless, but taken as a whole they describe a precise clinical entity. One would not have picked up a D trisomy, G trisomy, Down's, Kleinfelter's, or Turner's syndrome using these criteria.

Peripheral blood samples were taken from the 20 patients selected for study. A terminal labeling autoradiographic technique was used. Tritiated thymidine H^3-TdR, specific activity 1.9 c/mM, in a final concentration of 0.5–1.0 μc/ml, was added to phytohemagglutinin–stimulated leukocyte cultures after 64–68 hours of incubation at 37°C. Colcemid was added 3–4 hours before termination of the culture, after approximately 72 hours of incubation. Metaphase figures were photographed before and after the application of Kodak AR-10 stripping film, as described by Bader, Miller, and Mukkerjee (27). The slides were exposed for 8 days. Karyotypes were made according to the Denver classification (28).

Results

Twenty patients were selected for chromosome analysis. Two females were found to have a partial deletion of the short arm of a B group chromosome. Two males appeared to have a very small deletion of the short arm of a 4/5 chromosome, but the deletion is much too small to be proven; one female was found to have a 21 or 22 trisomy; and 15 patients had no identifiable chromosomal abnormality.

Autoradiography was performed on the two patients found to have a partial deletion of the short arm of a 4/5 chromosome. The B group chromosome with the partial deletion of the short arm was lightly labelled with silver grains, and is therefore the earlier replicating chromosome ※5. Although the autoradiographic studies on the patient with G group trisomy were inconclusive, it is felt that since this patient was picked up in a screening for first arch abnormalities and does not have the signs of Down's syndrome, that she is a 22 trisomic (29).

The fact that patients were found who either had no chromosomal abnormality, or else had a partial deletion of the short arm of chromosome ※5, while screening with features thought to be due to a partial deletion of chromosome ※4, casts some doubt on the specificity of the clinical features thought to be due to a partial deletion of the short arm of chromosome ※4. To test this further, the clinical features of these two patients having a partial deletion of the short arm of chromosome ※5 were compared with those of one patient known to have a partial deletion of the short arm of chromosome ※4. This case had previously been identified as a partial monosomy of chromosome ※4 by W. R. Breg, at the Southbury Training School in Connecticut (26).

Table I is a list of the clinical findings of the three patients studied. Patient A and patient B both have a deletion of a portion of the short arm of chromosome ※5, and patient C has a deletion of a portion of the short arm of chromosome ※4.

There is a large degree of overlap in the clinical features demonstrated by these three patients. This does not exclude the possibility that if a more distinctive feature of the syndrome, e.g., cat cry during the first week of life were present, that a more certain clinical diagnosis would be possible.

Another approach in comparing the two syndromes would be to look for genetic differences, i.e., differences in specific gene loci. One type of a genetic marker would be the presence of a recessive disease, which in these patients could be the result of the loss of a portion of the short arm of a B group chro-

TABLE I

Features	Patient A	Patient B	Patient C
Sex	Female	Female	Male
Age	50 years	54 years	15 years
I.Q.[1]*	21	18	7
Vocalization	Talks slightly	Minimal	None
Motor ability	Walks	Walks	Spastic dysplasia Sits
Height	Short stature (4'7" = 139 cm)	Short stature	Short stature
Microcephaly[1]	19.5" = 49.5 cm	+	+
Rounded facies[1]	+	+	+
Flat nasal bridge[1]	+	+	+
Small beaked nose[1]	+	+	+
Micrognathia[1]	+	+	+
Hypertelorism[1]	+	++	+
Epicanthus	+	+++	0
Cataract	0	Right, congenital	0
Strabismus	0	0	External, alternating
Nystagmus	0	0	0
Simple, low set ears[1]	+	+	+
Arch of palate	High	Intermediate	High
Genitalia			First degree hypospadias
Hands:			
Tapering of fingers	+	+	+
Bilateral clinodactyly of 5th digit	+	+	0
Short metacarpals	+	0	0

*[1] = The high incidence of these features is the result of using these characteristics as criteria in selecting the population.

mosome. The genes located on the corresponding part of the homologous chromosome would therefore show pseudodominance for the recessive disease. The recessive disease could also be due to homozygosity of the patient for the disease. To demonstrate the latter, a marker for the carrier state must be recognized in order to identify the heterozygous condition in both parents.

A long list of diseases transmitted by an autosomal recessive gene were studied (30), and those diseases which could be easily and accurately identified by someone who is not an expert opthalmologist or radiologist were chosen. The three patients were tested for 40 of the most easily demonstrable autosomally transmitted recessive diseases (see Table II).

Patient B was found to have a congenital cataract, which may be inherited as an autosomal recessive. Hospital records of the same patient indicate that she was suspected of having ectopia lentis, which is also inherited as an autosomal recessive. In the literature, one case of a deletion of the short arm of chromosome #4 also had congenital cataract (20), and two cases have been reported having pupillary ectopy (20, 25) which is transmitted as an autosomal recessive trait. Patient A had a double row of eyelashes, which may be inherited as either a dominant or recessive characteristic. Patient C had no autosomal recessive traits.

Thus, the evidence for a locus of a recessive disease on chromosome #4 or #5 is not conclusive, and further investigation is required. A better method might be to study the chromosomes of a patient having an autosomal recessive disease with other abnormalities, to determine if there is a chromosomal deletion.

Conclusions

1. It is difficult to differentiate the cri du chat syndrome from that associated with a partial deletion of chromosome ✕4 by using clinical criteria. It

TABLE II

Diseases Transmitted by an Autosomal Recessive Gene

Identifying Number of the Disease as Given by McKusick (30)	Name of Disease	Patient A	Patient B	Patient C
	Diseases of bone, development:			
2004	Achondrogenesis—shortening of limbs, hypoplastic digits, each digit containing only one segment of bone	0	0	0
2006	Acrocephalopolysyndactyly—Brachydactyly and syndactyly in hands, and preaxial polydactyly and syndactyly of toes	0	0	0
2067	Bifid nose	0	0	0
2098	Cleft lip, median, associated with postaxial polydactyly	0	0	0
2113	Craniosynostosis with radial defects	0	0	0
2114	Cretinism	0	0	0
2151, 2152	Dwarfism	0	0	0
2309	Leprechaunism	0	0	0
2409	Osteoetrosis (marble bones), macrocephaly, progressive deafness and blindness	0	0	0
2419	Peromelia with micrognathia	0	0	0
2445	Pycnodysostosis—deformity of skull (wide sutures), maxilla and phalanges (acro-osteolysis), osteosclerosis, and fragility of bone	0	0	0
	Eye defects:			
2050, 2051	Anopthalmus	0	0	0
2080	Cataract, congenital	0	+	0
2157	Ectopia lentis	0	?	0
2158	Ectopia lentis associated with ectopia of pupil	0	0	0
2347	Micro-opthalus	0	0	0
	Blood diseases and amino acidemias:			
2014	Congenital afibrinogenemia	0	0	0
2015	Agammablobulinemia	0	0	0
2243, 2266	Hydroxyprolinemia	0	0	0
2259	Hyperlysinemia	0	0	0
2261	Hypermethioninemia	0	0	0
2341	Methemoglobinemia due to deficiency of methemoglobinemia reductase (diaphorase)	0	0	0
2421	PKU	0	0	0
2516	Tyrosinemia	0	0	0
2519	Valinemia	0	0	0

TABLE II—*Continued*

Identifying Number of the Disease as Given by McKusick (30)	Name of Disease	Patient A	Patient B	Patient C
	Amino acid urias and excess sugars in urine:			
2097	Citrullinuria	0	0	0
2134	Cystinuria—homozygote excretes large amounts cystine, lys, arginine, orn in urine. Heterozygote demonstrates no abnormal amino acid urias	0	0	0
2190	Frusctosuria	0	0	0
2237	Homocystinuria	0	0	0
2246	Hyper-B-alaninemia, excess B-amino acids (B-ala, B-amino-isobutyric acid and taurine) in urine	0	0	0
2329	Maple syrup urine disease	0	0	0
2409	Oxalosis	0	0	0
2416	Pentosuria (L-xylulosuria)	0	0	0
	Diseases of the skin:			
2055	Aplasia cutis congenita	0	0	0
2065	Atrichia with papular lesions	0	0	0
	Miscellaneous diseases:			
2017–2020	Albinism	0	0	0
2033	Alopecia, epilepsy, oligophrenic syndrome of Moynihan	0	0	0
2149, 1209	Double row of eyelashes (possible dominant or recessive inheritance)	+	0	0

is possible that the presence of any of the following features would indicate a partial deletion of chromosome ※4, since they have never been reported in the cri du chat syndrome: pupillary ectopy; coloboma of the iris; exopthalmus; dislocation of the hip; hypospadias; epileptic signs; dysplasia of skin ridges; presence of volar pads; and midline scalp defects (25, 31).

2. The evidence for a locus of a recessive disease on the short arm of chromosomes ※4 or ※5 is not conclusive.

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CLINICO-PATHOLOGICAL CONFERENCE

Pulmonary Cavities

Edited by

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A 55-year-old housewife was admitted to the City Hospital Center at Elmhurst because of stupor.

Thirteen months earlier she experienced tenderness and swelling of both ankles, associated with tender nodular areas over both tibia. Shortly thereafter, she noted an ulcerated area above her left ankle. She felt irritation in her left eye 3 weeks later. The following week she noted mild left posterior chest pain. She also had stiffness and pain in her fingers. She was admitted to The Mount Sinai Hospital after a marginal ulcer was seen in her left eye. She had previously had a right oophorectomy and salpingectomy at age 38 for a ruptured ovarian cyst, and phlebitis of the lower extremities during pregnancy. She had lost 45 pounds in the past year, but attributed the loss of weight to dieting. She smoked one pack of cigarettes per day. She previously lived in Indiana. There was no history of allergies, diabetes mellitus, or cardiac disease.

She was a well-nourished white woman. The blood pressure was 145/75, pulse 80/minute, and respirations 14/minute. Xanthelasmas were present on the upper eyelids, and a corneal ulcer was noted in the left eye. There were medium to coarse rales at both lung bases posteriorly. The heart was normal. The tip of the spleen was felt with deep inspiration. Arthritic deformities were present in the terminal phalanges of the fingers, and there were mild varicosities of both lower extremities. A 4–5 inch ulcer with an erythematous base was present over the left ankle.

A tuberculin test (first strength) was positive. The hemoglobin was 10.3 gm%, white blood count 11,700/mm^3, with a shift to the left, and erythrocyte sedimentation rate 129 mm/hour. The blood urea nitrogen, fasting blood sugar, serum electrolytes, calcium, phosphorus, uric acid, SGOT, and alkaline phosphatase activity were normal. The serum albumin was 43.5%, alpha$_1$ globulin 5.8%, alpha$_2$ globulin 16.9%, beta 12.2%, and gamma 21.2%. The urine contained moderate amount of protein and red blood cell casts. A Casoni skin test was negative. Biopsies of the skin, muscle, and ulceration of the leg were nondiagnostic. Cultures of sputum, gastric washings, the leg ulcer, and the bone marrow failed to reveal pathogenic organisms. An x-ray of the chest showed two large cavities. In the right upper lobe, an air-fluid level was seen in one of the cavities (Fig. 1). An x-ray examination of the sinuses showed diffuse opacification of both maxillary antra. There was no evidence of destruction of adjacent bone. There appeared to be minimal haziness of the sphenoid sinus. The ethmoid and frontal sinuses appeared normal.

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Four months later she was readmitted to The Mount Sinai Hospital for treatment of scleromalacia perforans. Seven months prior to admission she reentered The Mount Sinai Hospital with headaches and an increase in the pain in the eye. The blood pressure was 170/80. There was clubbing of the terminal phalanges. Her spleen was palpated two fingerbreadths below the left costal margin. The following day she experienced severe dyspnea and chest pain, and was treated with hyperbaric oxygen. Shortly thereafter, hematemesis developed. A gastrointestinal series showed an ulcer of the greater curvature of the stomach.

On the day prior to admission to the City Hospital Center at Elmhurst, she experienced an occipital headache. The following day she noted right facial weakness and weakness of the right side of her body.

She was stuporous on admission. The blood pressure was 200/100, temperature 97°F, and pulse 84/minute. Within 6 hours she became comatose with flaccid quadriplegia. A spinal tap revealed xanthochromic fluid. The opening spinal fluid pressure was 335 mm H_2O, and the spinal fluid contained 52 mg% of protein, 64 mg% sugar, and red blood cells 4,000/mm^3. The hemoglobin was 12.5 gm%, and white blood cells 14,600/mm^3, with 93% polymorphs, 5% lymphocytes, 1% monocytes, and 1% eosinophils. The urine contained marked protein and a few red blood cells and white blood cells.

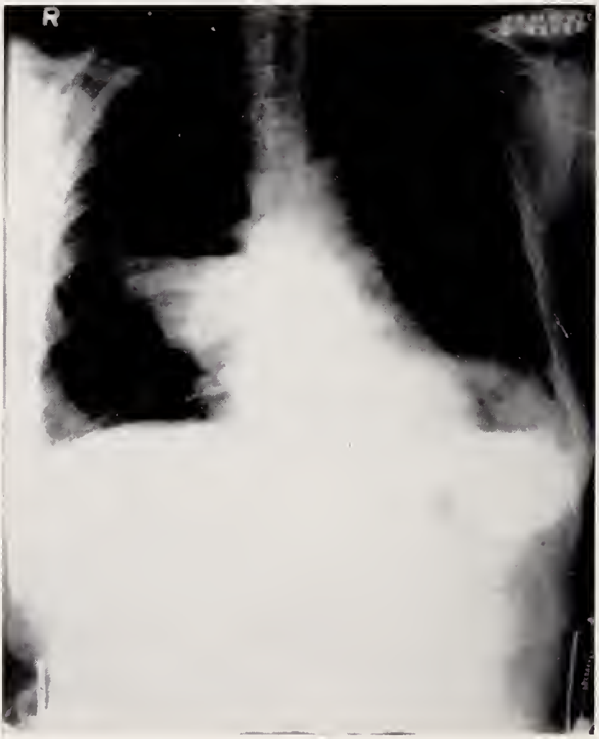

Fig. 1. Chest x-ray.

Intravenous mannitol was begun, and Aramine® was added to control shock, but the patient died the day following admission.

Dr. H. W. Berger:* The patient was a 55-year-old white woman who initially had physical findings compatible with erythema nodosum, consisting of tender nodules over the anterior part of the legs, and arthritis involving the ankles. Erythema nodosum is a syndrome of unknown etiology thought by many to be related to hypersensitivity, and occurring in response to a large number of stimuli. It is seen most commonly following streptococcal infections, but it is also frequently a manifestation of hypersensitivity to drugs.

Erythema nodosum is particularly interesting because it is an early manifestation of sarcoidosis when found in association with bilateral hilar nodes. In patients presenting in this fashion, there is usually complete resolution of the enlarged hilar nodes within 2 years. It is also seen in tuberculosis, various fungus infections (particularly coccidioidomycosis and histoplasmosis), as well as in ulcerative colitis, and some of the connective tissue diseases.

The clinical spectrum of erythema nodosum consists of several components. The first is tender, red, erythematous nodules on the anterior surface of the legs which usually heal completely and, at most, leave some pigmentation. The second component of the syndrome is arthritis, which most commonly involves the ankles but also may involve the knees or other joints. These patients usually have fever and may have an episcleritis. In those patients who have involvement of the joints without readily visible nodules, rheumatic fever may be mistakenly diagnosed.

It appears that this patient may have had erythema nodosum as one of the first manifestations of her illness. Of importance was the ulceration of the ankle. It was present in an area where there was minimal venous insufficiency. When the ulcer failed to heal, it became apparent that it was probably related to some underlying disease, possibly a vasculitis. However, no evidence of vasculitis could be found on biopsy. This is not unusual because pathologists frequently have difficulty in making a definite diagnosis from the small amount of tissue obtained by biopsy.

The major clinical manifestation of her illness was the striking involvement of the eye which failed to heal. Initially, an ulceration was seen which eventually developed into scleromalacia perforans, which refers to a thinning of the sclera with eventual extrusion of the uveal tract through the sclera. Scleromalacia perforans is thought to be a manifestation of hypersensitivity, and is found in connective tissue diseases, particularly rheumatoid arthritis. The patient had some evidence of arthritis of the fingers, but the exact type is not clear from the protocol, except that it was different from the arthritis which is usually associated with erythema nodosum.

She also had a loss of 45 pounds of weight, which I think reflects the systemic nature of her disease. Patients will frequently claim that they have lost weight because of dieting, but usually this is a denial of symptoms.

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It is important that she had no history of allergies, since many patients with connective tissue disorders have a history of allergy to drugs, or have a history of asthma or hayfever early in life.

The blood pressure was normal until she suffered a subarachnoid hemorrhage. The tip of the spleen was felt, as it is in many patients who are diagnostic problems. In this particular case it was probably a reflection of a generalized disorder, but the enlarged spleen was not particularly helpful diagnostically.

The tuberculin test was positive, which is not surprising since the majority of patients in this age group have been exposed to tubercle bacilli. She had anemia and eosinophilia. Eosinophilia, as you know, is found frequently in patients who have hypersensitivity diseases, and particularly in polyarteritis nodosa, one of the conditions that was considered. Eosinophilia, however, is unusual in Wegener's granulomatosis.

She had evidence of glomerulitis manifested by proteinuria, red cells, and red cell casts in the urine, and this was the first clue suggesting a connective tissue disorder.

The x-rays of the sinuses showed clouding, a finding of Wegener's granulomatosis, but clouding of the sinuses in a person of this age is not unusual. Erosion of bone would lend more support to a diagnosis of Wegener's granulomatosis. An x-ray examination of her chest, taken fairly early in the course of her illness, revealed two striking abnormalities. There was a very sharply demarcated mass with a thick, irregular nodular wall, and a fluid level in the superior segment of the right lower lobe, and a similar lesion in the left lower lobe.

She had no pulmonary symptoms, except for a nonproductive cough. In fact, she was relatively well in spite of the presence of these large cavitary lesions. Purulent abscess cavities are usually associated with cough, purulent sputum, fever, and a foul odor. The border of abscess cavities tend not to be as sharply defined as those in this patient. In addition, she was given several courses of antibiotics without any improvement or change in the size of the cavities. Certain specific organisms may produce a suppurative pneumonia such as Staphylococcus and Klebsiella, but with suppurative pneumonia, the abscesses are frequently multiple, as distinguished from putrid lung abscess which is usually single. I don't think that we have any evidence that our patient had a lung abscess.

Any patient having a cavity within the lung, regardless of location, should be suspected of having tuberculosis. Characteristically, tuberculosis produces bilateral upper lobe disease. The tuberculous cavities usually have a thin to moderate wall, and fluid levels are uncommon. However, if fluid is present, the fluid level is very small. Tuberculosis is predominantly found in the upper lobes posteriorly, and often shows bronchogenic spread. Our patient was studied for tuberculosis, but the smears and cultures did not grow tubercle bacilli. Tuberculosis may not always be easily diagnosed. Tuberculous cavities also occur in the lower lung field and occasionally have characteristics that are unusual, so that tuberculosis must be considered as a possible diagnosis in any patient who has a cavity, regardless of its location, and regardless of its appearance. In lung cancer, cavities occur from breakdown of the cancerous mass, or from breakdown of an area of pneumonia behind bronchial obstruction. Although the x-ray picture of one lesion is similar in appearance, it would be unusual for cancer to be found in two locations. In addition, a large fluid level is uncommon.

Occasionally, an area of pulmonary infarction cavitates. This diagnosis was considered in the patient since she had a history of phlebitis. Again, two cavities in a patient with pulmonary infarction would be uncommon. Polyarteritis and Wegener's granulomatosis can produce lesions in the lung. The lesions of polyarteritis may be nodular and cavitate. The patient may also have transient infiltrations and pleural effusions. In Wegener's granulomatosis, the cavities are found mainly in the lower lobes. They may be very large and lack areas of surrounding infiltration. The cavities in our patient were consistent with the diagnosis of Wegener's granulomatosis.

The x-ray abnormality was a very striking feature of this case, and I think that we would best consider this case as one showing evidence of ulceration both in the eye and in the lower extremities, a glomerulitis, and cavitating lesions in the lungs. The clinical diagnosis that I considered prior to her death was Wegener's granulomatosis. Wegener's granulomatosis is usually grouped with the connective tissue diseases. Many feel that it is a variant of poly-

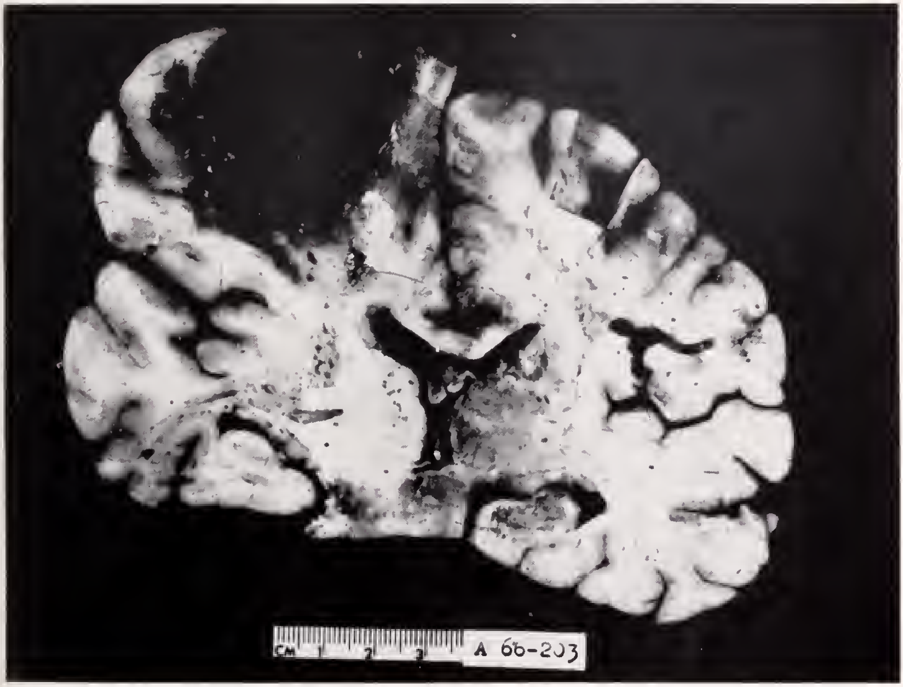

Fig. 2. Brain (gross), showing extensive left-sided intracerebral hemorrhage.

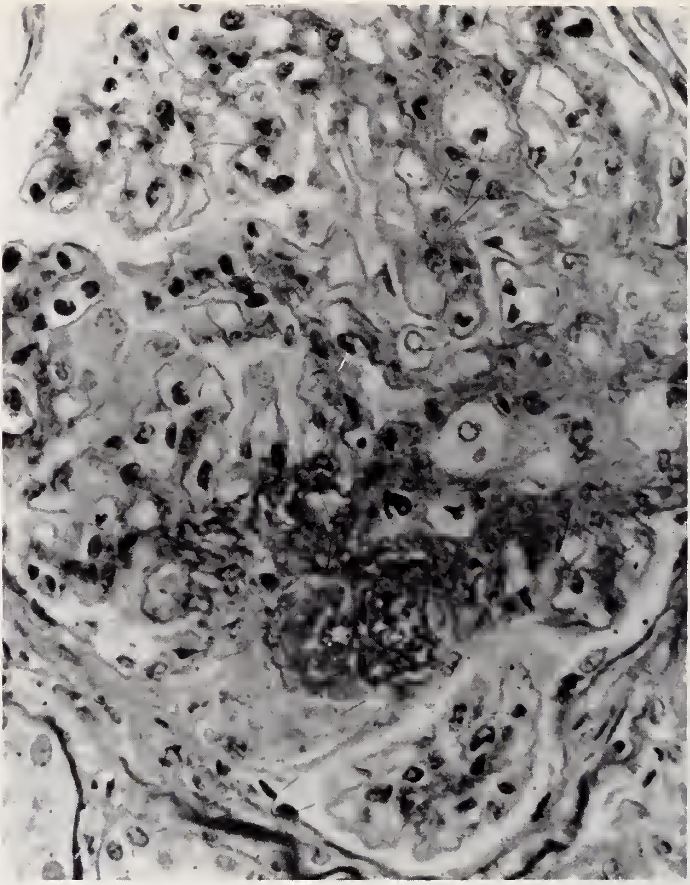

Fig. 3. Kidney (microscopic, PAS), showing focal necrotizing glomerulitis.

arteritis nodosa. It consists of a clinical triad of necrotizing granulomas of the upper and lower respiratory tract, glomerulitis, and vasculitis.

The granulomas in the upper respiratory tract are frequently the presenting symptom, and they may lead to a purulent rhinorrhea. Patients may develop nasal erosions which may communicate with the orbit or the face, and can be disfiguring. The granulomas in the lower respiratory tract may produce cavitary lesions. The disease is found equally in men and women, usually in middle age. Commonly, the patients have no allergic background, and they usually do not have eospinophilia, although some feel that it may occur more frequently than reported.

Our patient was somewhat unusual in that she had eosinophilia which persisted throughout the clinical course. Involvement of the sinuses is fairly characteristic. Destruction of the sinuses with fistulae leading into the orbit, or externally, is more suggestive of the disease than purulent discharge and clouding of the sinuses. Pulmonary involvement occurs commonly, and consists of nodules, infiltrations, and pleural effusions. The nodules frequently cavitate.

This disease is found predominantly in the lower lobes, and the cavities have a tendency to be very large.

The involvement of the kidneys consists of a glomerulitis. Eventually, most of the patients experience hypertension and usually die with uremia. Arthritis is a common accompaniment of Wegener's granulomatosis, as it is with other diseases of connective tissue. Ulcerations also are common, and have a tendency not to heal due to the vasculitis. Certainly, one of the earliest indications in our patient that she might have had a vasculitis was that she had an ulceration on her leg which did not heal in spite of the good medical management. The eyes are commonly involved in Wegener's and may lead to scleromalacia perforans.

The prognosis is poor. Most of the patients die within a year or two of onset of their disease, usually from renal failure. Several modalities of treatment have been tried. Steroids are not particularly helpful. Two regimes that have shown some promise are a combination of Cytoxan® and Prednisone, and chlorambucil.

In summary, this patient had a clinical picture consisting of glomerulitis, cavitary lesions of the lung, and ulcerations of the eye and lower extremity compatible with Wegener's granulomatosis.

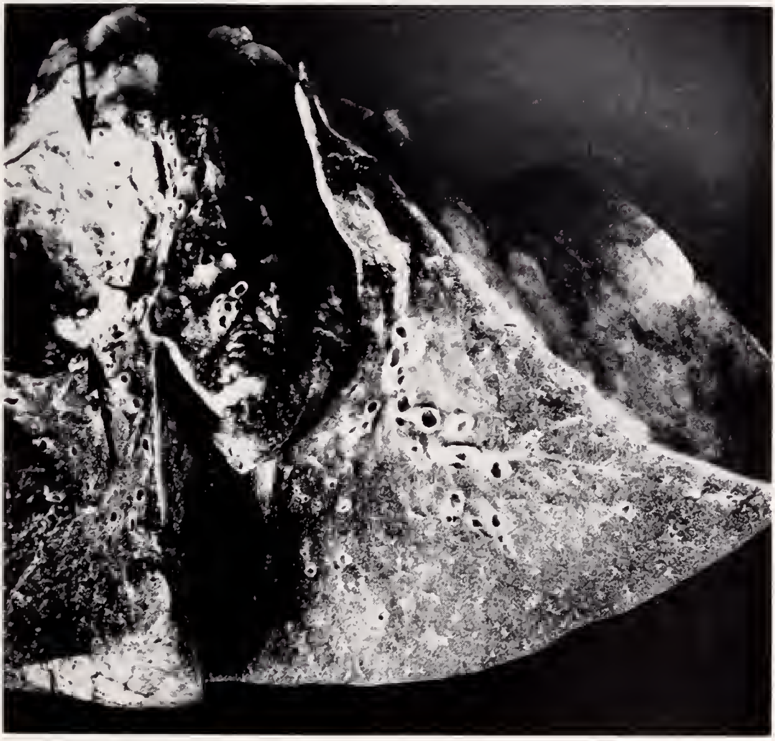

FIG. 4. Lung (gross), showing large cavity in the right upper lobe.

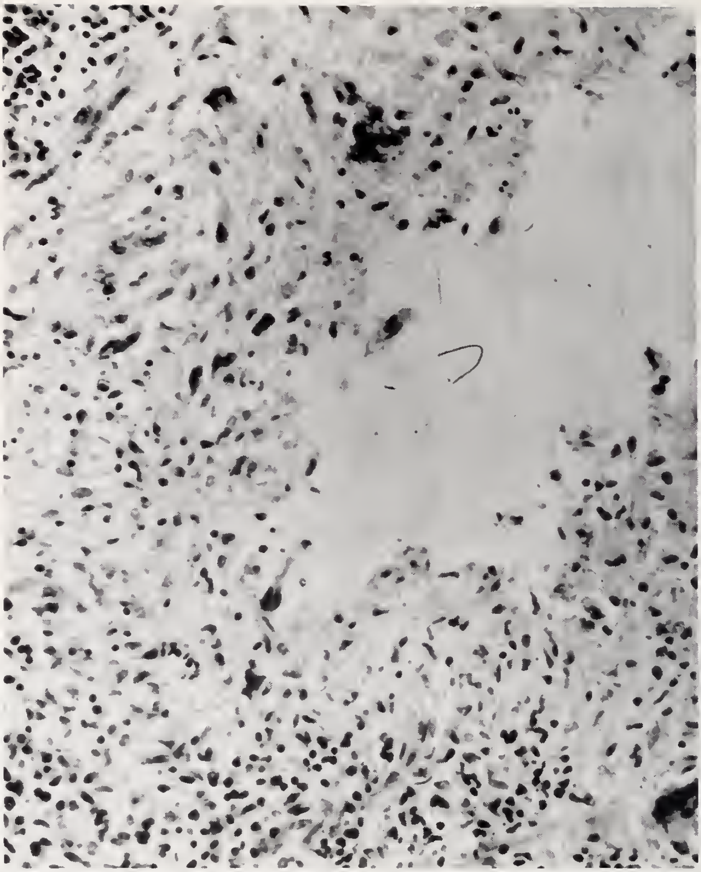

Fig. 5. Lung (microscopic, H & E) near edge of cavity, showing necrosis, acute and chronic inflammatory cells, and giant cells.

*Dr. H. H. Baruch**: The heart, spleen, and liver were normal. Examination of the eyes showed a chronic inflammatory process and changes as seen in scleromalacia perforans. Whether the inflammatory process was primary or secondary to perforation could not be determined. Scleromalacia perforans frequently occurs in so-called collagen diseases, and the pathological findings are not specific.

On sectioning the brain, there was a large intracerebral hemorrhage which had ruptured into the subarachnoid space (Fig. 2). Microscopically, there was destruction of brain tissue by the hemorrhage. No evidence of vasculitis was found in the cerebral vessels. The episode of gastrointestinal bleeding was due to a gastric ulcer. The blood vessels near the ulcer were surrounded by inflammatory exudate which, while a "vasculitis," probably represented a reaction to the ulcer.

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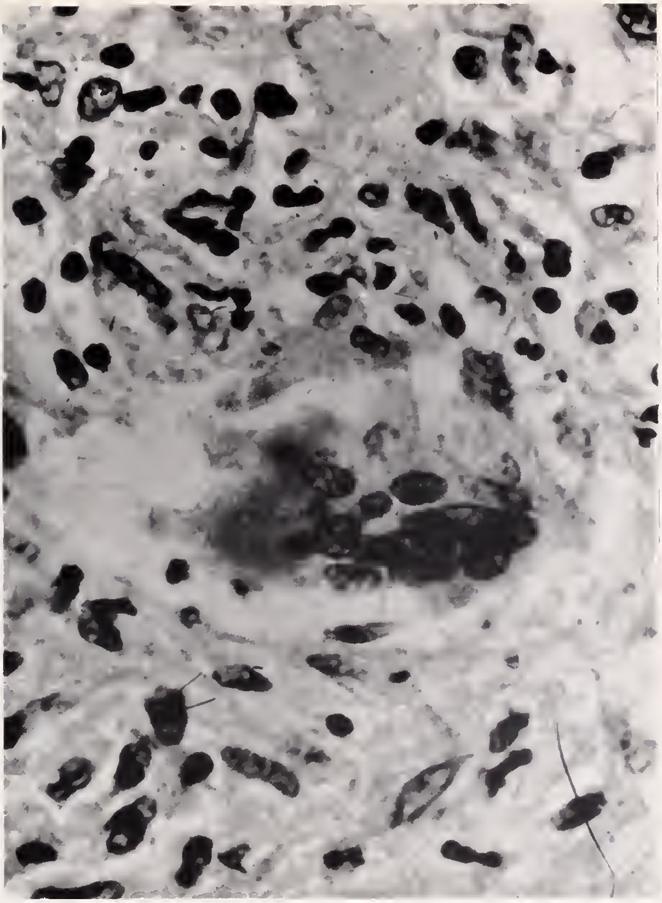

Fig. 6. Lung (microscopic, H & E), showing giant cell.

The kidneys appeared normal on gross examination. On microscopic examination, however, there was a "focal glomerulitis" (Fig. 3).

She had two huge cavities in the lungs. One was present in the left lower lobe, and the other in the right upper lobe (Fig. 4). Culture of the cavities failed to grow any organisms. The lining of these cavities was formed by granulation tissue with numerous fibroblasts and giant cells (Fig. 5). The nuclei of the giant cells were arranged in different patterns, sometimes resembling Langhans' cells (Fig. 6). Occasionally, a giant cell was seen around a vessel. We were not able to find convincing vasculitis.

In Wegener's granulomatosis, destruction of tissue is marked compared to the usual vasculitities. The three classical features usually described are the necrotizing giant cell granulomatous lesions of the respiratory tract, focal necrotizing glomerulitis, and necrotizing vaculitis, most marked in the lungs. Our patient had the first two features, but the latter was not convincing. However, cases of Wegener's granulomatosis have been accepted where it is absent, and it is disputed whether vasculitis must be demonstrated in order to make a diagnosis of Wegener's granulomatosis. Other diseases which may be confused with or related to Wegener's include the lethal granulomas, sarcoidosis, allergic granuloma, and periarteritis nodosa.

In summary, we considered that the patient had Wegener's granulomatosis, although it is not typical because of the absence of a demonstrable vasculitis. She had scleromalacia perforans, although this may have been the result of direct tissue destruction rather than having been due to a vascular lesion. This case, however, does illustrate one example of the spectrum of disease which involves blood vessels, respiratory tract, and kidneys. The relation between the kidneys and the lungs is rather an intriguing one, because in other diseases like Goodpasture's syndrome there is a similar association. Some believe that there is a hypersensitivity effect primary in the lungs which secondarily involves the blood vessels and kidneys. Therefore, if the illness is of short duration, the blood vessels may not be involved. Various studies to date have been performed in an attempt to demonstrate an antibody-antigen type of reaction in Wegener's granulomatosis. They have not been successful, and only fibrinogen had been found in the renal lesions. Investigations using immunofluorescent methods have been unable to show globulin or complement in the involved kidneys.

Question: Was the absence of a vasculitis due to the fact that the patient had been on steroids?

Dr. Baruch: Possibly if she had lived longer vasculitis might have developed. If it had healed, we should have seen destruction of the elastica.

Final Diagnoses:

1. NECROTIZING GRANULOMATOSIS (WEGENER'S GRANULOMATOSIS).
    - A. FOCAL NECROSIS WITH CAVITATION OF THE LUNGS (RIGHT UPPER LOBE AND LEFT LOWER LOBE).
    - B. FOCAL GLOMERULITIS OF THE KIDNEYS.
    - C. INTRACEREBRAL HEMORRHAGE, EXTENSIVE AND FRESH, OF THE LEFT FRONTAL AND PARIETAL LOBES.
    - D. SCLEROMALACIA PERFORANS OF THE LEFT EYE.
2. PEPTIC ULCER OF THE STOMACH, HEALING.

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OPHTHALMOLOGICAL NOTES

THEODORE W. LIEBERMAN, M.D., EDITOR

Roth's Spots

BY HARVEY H. SILVERBERG, M.D.

Clinicians frequently scan the ocular fundus for Roth's spots in suspected cases of bacterial endocarditis. Harrison's *Principles of Internal Medicine* describes the Roth's spot as a "circular or oval, pale area surrounded by a ring of hemorrhage, usually near the disc" (1). Roth, in his original paper on retinitis septica (1872), reported a series of patients with sepsis secondary to a variety of causes, in which there were small white spots and separate foci of hemorrhages (2). Litten (1878) in a later amplification of Roth's findings, gave the name Roth's spots to "round or elongated white foci occurring close to the nerve head . . . single or multiple and occasionally surrounded by a hemorrhagic ring" (3). Litten attached little diagnostic or prognostic value to these signs. It remained for Doherty and Trubek, 40 years later, to call attention to the "pathognomonic canoe shaped linear hemorrhagic spot with a light central area" associated with bacterial endocarditis (4). Although these authors emphasized that Litten actually described the white centered hemorrhages, the eponym Roth's spots has been generally adopted for these lesions. Perhaps the clearest description of the retinal picture in bacterial endocarditis is given by Friedberg: "Petechiae with and without white centers are also encountered in the retina. Often these petechiae have a characteristic boat-shaped appearance. Whitish lesions known as Roth's spots are observed both in acute and subacute bacterial endocarditis as in other diseases with severe anemia" (5).

Roth's spots usually present bilaterally at the posterior pole, but characteristically do not involve the macula. There is little or no visual impairment, making the name "silent retinopathy" apt (7). In addition to endocarditis, Roth's spots have been reported in severe anemias, leukemia, collagen disease, and a variety of other conditions including viral pneumonia, kala-azar, and phlebitis (6–7). In some of these other conditions, notably leukemia, visual loss may result from hemorrhages into the macula.

The histopathology of the Roth's spot has been studied extensively, with varying interpretations. Roth originally described retinal hemorrhages and ganglioform degeneration in the nerve fibre layer. Litten and others reported a homogeneous, structureless, hyaline-like material in the nerve fibre layer. Hogan and Zimmerman agreed with Roth, and ascribed the lesions to infarcts in the nerve fibre layer of the retina (cytoid bodies) (8).

On the other hand, many investigators have reported a primarily cellular response. Bacterial emboli have been found in many cases, but they have been

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of minimal virulence, exciting little or no tissue reaction. Dienst and Gartner described multiple foci of cellular infiltrates, chiefly around blood vessels (9). The cells were usually small round cells. They pointed out that although hemorrhages were more striking clinically, there were 5 to 6 areas of white cell infiltration for every area of hemorrhage.

Since the advent of the antibiotic era, the opportunity to study cases pathologically has been reduced. Kennedy and Wise recently reported a case in which they were able to correlate fundus and microscopic findings (10). Fundus photographs were taken on the day prior to demise in a patient severely ill with subacute bacterial endocarditis. They showed white centered elliptical hemorrhages in the posterior pole of each eye and one white spot without surrounding hemorrhage in one eye. At autopsy serial sections were made through each of these areas. The three white centered hemorrhages showed central aggregations of white cells. Hemorrhages were noted in the nerve fibre and outer plexiform layer. No cytoid bodies were observed. On the other hand, sections through the white spot showed an acellular area in the nerve fibre layer containing multiple cytoid bodies.

It thus appears that there are two distinct clinical and microscopic lesions corresponding to Roth's spots: white spots, and white centered hemorrhages. The white spots, as actually described by Roth, appear clinically as cotton wool exudates. These exudates histologically are aggregations of cytoid bodies, and they are considered to be infarctions of the nerve fibre layer. Cytoid bodies are seen in a variety of systemic conditions in which the retina is affected. It has been suggested that the cytoid body represents an attempt by a damaged axon to regenerate itself (11).

The white centered hemorrhage is a focal white cell accumulation with surrounding red blood cell diapedesis. The type of white cell probably depends on the age of the lesion. Polymorphonuclear leukocytes predominate in the acute stage, and round and mononuclear cells appear in the older lesion. An attractive postulate for these white centered hemorrhages is septic embolization with secondary endothelial damage leading to extravasation of red blood cells. The striking absence of tissue reaction does not support this theory. Alternatively, a hemorrhage may be the first event with secondary white cell infiltration. Vouters (7) has observed retinal hemorrhages which developed white centers within 8 hours, lending clinical support to this hypothesis.

Summary

The retinal findings in bacterial endocarditis include linear hemorrhages, white spots, and hemorrhages with white centers. Although Roth's spots are properly the white spots, popular usage has given the eponym to the white centered hemorrhages. These two lesions differ clinically and microscopically. Both are found in a number of other systemic diseases, and neither can be considered pathognomonic of bacterial endocarditis.

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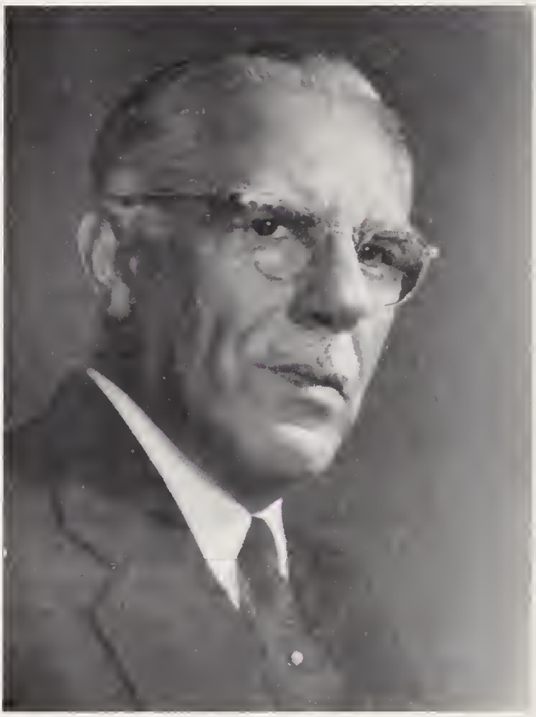
ANNOUNCEMENT

THE RALPH COLP AWARDS FOR 1969

A Ralph Colp Award for 1969 has been given to Dr. A. Robert Beck, and another to Dr. Franklin M. Klion. These awards are in recognition of the excellence of the departments in the Journal which each one has so conscientiously and meticulously conducted over a period of years.



This award is made by the Ralph Colp Fund, for the best paper published in The Mount Sinai Journal of Medicine by a member of the house staff or a junior member of the attending staff. Preference is given to papers on surgical subjects. The Fund was established in honor of Dr. Ralph Colp, distinguished surgeon and long-time Chief of Surgery of The Mount Sinai Hospital.

In Memoriam

WILLIAM DAMESHEK, M.D.
1900–1969

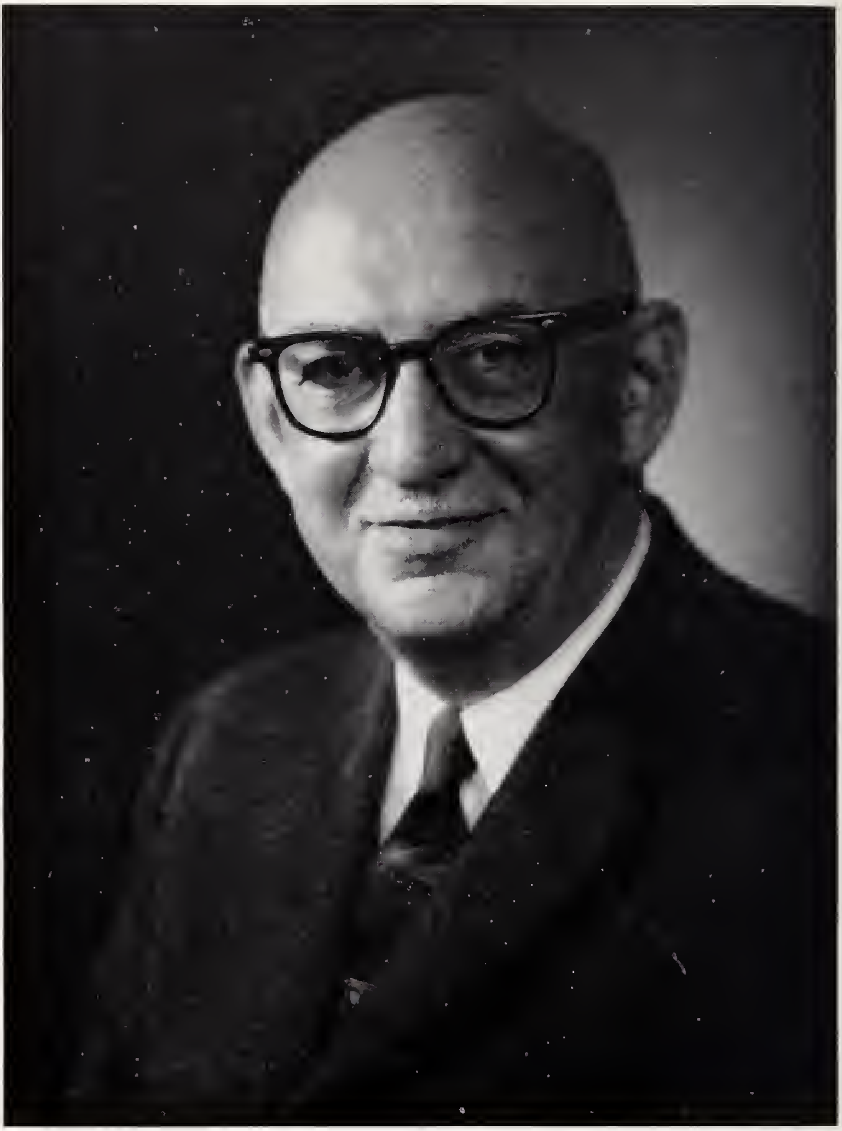
It is with great sorrow that we record the passing of Dr. William Dameshek on October 6, 1969, in his 69th year. Medical science has lost a great physician, hematologist, scholar, and humanitarian.

Dr. Dameshek's brilliant achievements and the recognitions and awards bestowed upon him by his colleagues and friends are too numerous to list fully here. At his death he was Emeritus Professor of Medicine at the Mount Sinai School of Medicine, Consultant Hematologist to The Mount Sinai Hospital, Editor-in-Chief of *Blood*, the international journal of hematology, and the Vice-President for Scientific Affairs of the Leukemia Society of America. He had come to The Mount Sinai Hospital after his retirement in 1966 as Professor of Medicine and Director of the Blood Research Laboratory of the Tufts-New England Medical Center in Boston. He was a founding member and past President of both the American Society of Hematology and the International Society of Hematology. He was the founder and Editor-in-Chief of *Blood*, the international journal of hematology, since its inception. He had authored more than 450 articles and books on hematology, including "The Hemorrhagic Disorders," "The Spleen and Hypersplenism," "Hemolytic Syndromes," and "Leukemia." He had received many medals and citations, including the Millard O. Thompson Gold Medal of the American Geriatrics Society in 1968.

Dr. Dameshek's contributions to hematology extend much further than his publications. He had personally taught many of today's outstanding hematologists, and there was hardly a city in the eastern or western hemispheres where he might travel and not be met by one of his former students. His editorial comments and speculations were, and still are, central to the understanding of many hematologic diseases. He pioneered the technique of sternal and iliac crest bone marrow aspiration, a procedure which has now become the cornerstone of immunologic diagnosis.

Born in Veronezh, Russia at the turn of the century, Dr. Dameshek came to the United States as an infant, and grew up in the suburbs of Boston. He attended Harvard College and Harvard Medical School, and graduated with honors at the age of 23. In 1922 he married Rose Thurman of Brookline, and pursued his postgraduate studies at the Boston City and Beth Israel Hospitals.

In 1939 he founded the Blood Research Laboratory at the newly formed Joseph H. Pratt Diagnostic Clinic, later to become the New England Medical Center Hospitals complex. During the early years after its inception, Dr. Dameshek became widely known for his clinical and experimental studies on the antibody nature of acquired hemolytic anemia. Under his guidance, the



WILLIAM DAMESHEK, M.D.
1900–1969

Blood Research Laboratory blossomed into one of the leading hematological, clinical, and research facilities in the country and contributed many new techniques and concepts to hematology, including those of the use of nitrogen mustard in treatment of hematologic disorders, the nature of hypersplenism, a unified conception of myeloproliferative disorders, and the use of immunosuppressive antimetabolites in the treatment of autoimmune disease.

All of Dr. Dameshek's research efforts were prompted by the desire to better serve his patients. Throughout his 30 odd years of clinical practice, he remained an astute clinician who often could be correctly guided by instinct when his colleagues foundered.

He was a born teacher, who loved to teach. On ward rounds at the New England Medical Center, and later at this hospital, he would often be seen surrounded by a retinue of medical students and house staff. A tall, balding man, with a fringe of grey hair, his bearing seemed to convey a curious combination of aristocracy and geniality. His students were devoted to him, and he to them.

A tireless worker, he fulfilled commitments to medical practice, journal editorship, guest lectures, and international travel that would have taxed any three other men. Between these activities and far into the night, he wrote endless pages of recently completed studies or newly arrived at speculations.

At the time of his death, he was in the midst of conceptualizing a unified hypothesis of the immunoproliferative disorders as the basic process underlying most autoimmune disease and certain forms of lymphomas. He was also directing his efforts toward the achievement of clinical bone marrow homotransplantation in aplastic anemia and leukemia patients. For those of us who were personally acquainted with Dr. Dameshek, this has been a most grevious loss; we have been deprived of our teacher and our friend. For those who might have comprised his next generation of students, a great deal may still be learned of him by examining his writings. But the great man, physician, and teacher is gone.

Robert N. Taub, M.D.
for the
Editorial Board

Surgical Approaches to Midline Lesions at the Base of the Skull: A Review

ROBERT E. DECKER, M.D., AND LEONARD I. MALIS, M.D.

Midline lesions at the base of the skull have been considered in the past to be inoperable, partially resectable, and even when resectable, unpleasantly difficult. Accounting for the above has been the poor visualization of minimal surgical exposures, copious bleeding, and lack of adequate fluoroscopic depth monitoring. These built-in difficulties may prove to be a fertile ground for microsurgery. Presently, the use of the surgical dissecting microscope, portable image intensifiers, and specifically designed microinstruments has enabled experienced microneurosurgeons to avoid some of the earlier pitfalls in this region.

Title to surgery in this area has been claimed by many surgical subspecialists. Among these are ophthalmologists, otolaryngologists, head and neck surgeons, and orthopedic surgeons, as well as neurologic surgeons. Since regional microsurgery may some day develop as a subspecialty of its own, there certainly is need for a team approach to the base of the skull. It would seem to the authors that the neurosurgeon should be primarily responsible for surgery in this area for the following reasons:

(1) Intimate involvement with the establishment of the diagnosis and the location of lesion.

(2) Experience with the delicate handling of neural tissue in limited surgical exposures.

(3) Complications frequently involve neural structures.

Intimate knowledge of the anatomy of the paranasal sinuses, rhinoseptal region, oropharyngeal and deep cervical tissue planes, as well as the upper cervical spine and base of the skull is paramount for successful surgery in this region. Formal surgical exercises on fresh cadavers has been fruitful in satisfying this requirement.

The sphenoid sinus is the gateway to the sella and other structures of the base from many different external approaches. Therefore, detailed knowledge of the anatomical development and the variations of the sinus is useful (1). It is the first paranasal sinus to complete full development. Though nonexistent below primates, it is more or less developed in primates while reaching its fullest development in man. Pneumatization of the body of the human sphenoid is accomplished before 4 years of age, but continues, and development progresses until adult size is reached in the 12th year. There is further molding and reshaping throughout adult life. The sinus often varies beyond limits of the sphenoid body in different directions, forming recesses. The extensions are frequently anteriorly into the clinoid processes and optic foramen margins or

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laterally into the lesser wing of the sphenoid, rather than posteriorly or inferiorly. Vidic and Stom (1) found the dorsum sella and posterior clinoid processes completely pneumatized in less than 3% of 500 skulls. Hammer and Radberg (2) categorized the sphenoid sinus into the following types:

(1) *Presellar* (12%): Does not extend beyond the vertical plane of the tuberculum sella vertical to the orbital meatal plane.

(2) *Sellar* (85%): Pneumatization extends posteriorly beyond this vertical plane, and thus can be subdivided into four types based on further extension into the dorsum sella and posterior clinoid processes (1).

(3) *Concha* (3%): Absence of pneumatization, frequently associated with multiple congenital anomalies.

Surgery through a nonpneumatized sphenoid is controversial, but has been accomplished successfully with the use of highspeed air drills (3–5).

Early Development

The pioneers of the approach to the base of the skull were the great unspecialized surgeons of the early twentieth century (Table I). They were in search of an entry to the sella turcica for the prime purpose of removing pituitary neoplasms. Their methods were developed by the extensive use of cadavers in anatomical dissections. Giordano, in 1897, first suggested a superior nasal approach to the pituitary gland (6). This involved an osteoplastic resection of the anterior wall of the frontal sinus, entering the nasal cavity after opening into the ethmoid cells. From here the dissection was extended into the sphenoid sinus and then the floor of the sella. Though a short and direct method to the sella, it was quite radical and bloody. Many years later this procedure was modified as the transethmoid approach by Bateman and James, and then by Montgomery (7).

In 1898, Koenig (8) developed an oronasal procedure which consisted of separating the mucoperiosteal covering from the palatal process of the superior maxilla, and then splitting the maxilla and retracting the lateral halves. He then followed with a standard intramucosal approach to the vomer and then the sphenoid sinus. In 1900, Loewe (8) proposed a method that utilized a vertical paramedian nasal splitting incision, which also involved entering the maxillary antrum. He then developed it into a bimaxillary approach consisting

TABLE I

Pioneers of Transsphenoidal Route to Hypophysis

Name	Date	Procedure
Giordano	1897	Superior nasal fronto-ethmoid-sphenoid
Koenig	1898	Infranasal maxilla splitting
Loewe	1900	Paranasal maxilla splitting
Schloffer	1907	Superior-lateral nasal reflection
Kanavel and Halstead	1909 1910	Oronasal-septum reflection

of a large opening through the maxillary antrum, ethmoids, and nasal cavity before encountering the sphenoid. In 1907, Schloffer (8) successfully removed a tumor by a similar method. He retracted the entire nose inferiorly and to the right, and then excised the turbinates, bony septum, and the medial orbit to the optic foramen, with extensive removal of the maxillary sinus and ethmoids, to enter the sphenoid sinus. The above procedures did not gain popularity because of the entensive tissue destruction and resulting facial disfigurement.

In 1909, Kanavel (9) developed a midline infranasal approach to the pituitary that was soon adopted by Halstead (8), and later modified by Cushing. He incised the maxillary gingiva transorally with elevation of the mucoperiosteum on the maxilla, and then retracted the cartilaginous septum to one side. He removed the middle and inferior turbinates bilaterally, then lifted the mucosa from the vomer, resected the midline septum, and entered the sphenoid sinus. Cushing (10) modified this approach somewhat by removing the lower third of the cartilaginous septum after retracting the mucosa from the midline bilaterally. This extra mucosal approach was then extended to the bony sphenoid floor. He also developed a retractor to hold the mucosa laterally and, therefore, deterred copious bleeding from the site. Hirsch (11) adopted a similar approach, using an endonasal rather than an oronasal exposure. Thus, by 1910, the transsphenoidal approach to the pituitary gland was definitely established.

Modern Surgical Approaches

Since the original work of Cushing and Hirsch, there have been many modifications in the original approaches to the base of the skull (Table II). The pituitary is not the only target area at the present time. In the 1950's and early 1960's, Scoville (12), Southwick and Robinson (13), and Fang and Ong

TABLE II

Surgical Approaches to Base of Skull

Procedure	Name
Oronasal rhinoseptal to sella and upper and mid-clivus	Cushing, Guiot[x]*, and Hardy[x,m]*
Endonasal rhinoseptal to sella	Hirsch
Oro-antral-nasal to sella	Hamberger
Transantral-nasal to sella	Svien[x]
Transethmoid-sphenoidal to sella	Bateman, James, and Montgomery[m]
Transnasal osteoplastic to sella	Macbeth
Transnasal cavity approach to sella	Rosin and Lehrer[m]
Transoral to low clivus, C1 and C2	Scoville, Southwick, Robinson, Fang, and Ong
Transoral to mid- and low-clivus	Mullan and Yasargil[m]
Transcervical transclival	Stevenson[m], Fox[m], and French[m]
Oronasal rhinoseptal to tuberculum sella and planum sphenoidale	Decker[x,m]

* x = radio fluoroscopic control; m = microsurgery.

(14) used a transoral approach to the high cervical spine and low clivus for treatment of tumors of the cervical bodies, atlantoaxial dislocation, and tuberculous involvement of the region. Recently, neurosurgeons have become interested in an approach to the entire retroclival area for the purpose of removing benign tumors between clivus and brain stem (15–17). Now, with the further impetus of training in microvascular surgery, the retroclival vascular structures have been brought into striking distance.

Technical improvements in Cushing's oronasal rhinoseptal approach to the pituitary have been made by Guiot (18), and Hardy (5). Introduction of radiofluoroscopic control by Guiot, and the addition of microsurgery to the procedure by Hardy, has certainly changed the perspective of, and raised the horizens for, future accomplishments by this exposure. This same rhinoseptal approach can be used to attack tumors in the region of the sphenoid and upper and midretroclival regions. Extending the approach anteriorly, it is possible to expose the anterior communicating artery after removing the roof of the sphenoid sinus in the presellar region.

Various other approaches to the sphenoid sinus, and hence the sella, have been devised using both external and transoral incisions (Fig. 1). Svien (19) prefers a rather long, oblique route to the hypophysis, by extending a paranasal incision over the maxillary antrum, thus converting the maxillary antrum and nasal cavity, including the ethmoids, into one orifice. Hamburger (20) uses a similar exposure, entering the maxillary antrum by a Caldwell-Luk incision through the oral mucosa. This apparently increases the maneuverability of instruments at the surface. Bateman, and later James and Montgomery (7), have preferred the short oblique transethmoid route to the hypophysis. The incision is placed in the medial orbit and, therefore, the nasal cavity is entered via the lamina papyracea and ethmoid sinuses. This appears to be a worthwhile and versatile approach in their hands. Macbeth (4) has chosen an osteoplastic elevation of the skin and nasal bones to enter the sphenoid sinus in a short, midline exposure. The deficit of this procedure in the hands of less than expert plastic surgeons would seem to be that of a cosmetic defect. It also appears to be a more complex external procedure than necessary. A transnasal cavity approach has been utilized by Rosin and Lehrer (unpublished data), both alone, and in conjunction with, neurosurgeons. It involves the performance of bilateral, lateral rhinotomies, and reflection of the septum and columella, with the option of removing the middle and inferior turbinates if more extensive exposure is necessary. The cosmesis of this approach is certainly adequate, and exposure obtained in several cases has been excellent.

We favor the oronasal rhinoseptal approach as modified by Hardy because it is a straight midline approach, and it is helpful in anatomical orientation and receptive to televised radiofluoroscopic control. In addition, it is an extramucosal approach and, therefore, it may give less incidence of postoperative infections in a large number of cases. However, it is notable that even the numerous transmucosal approaches, as illustrated above, have a very low incidence of wound infection. Responsible for the low infection rate, is probably the

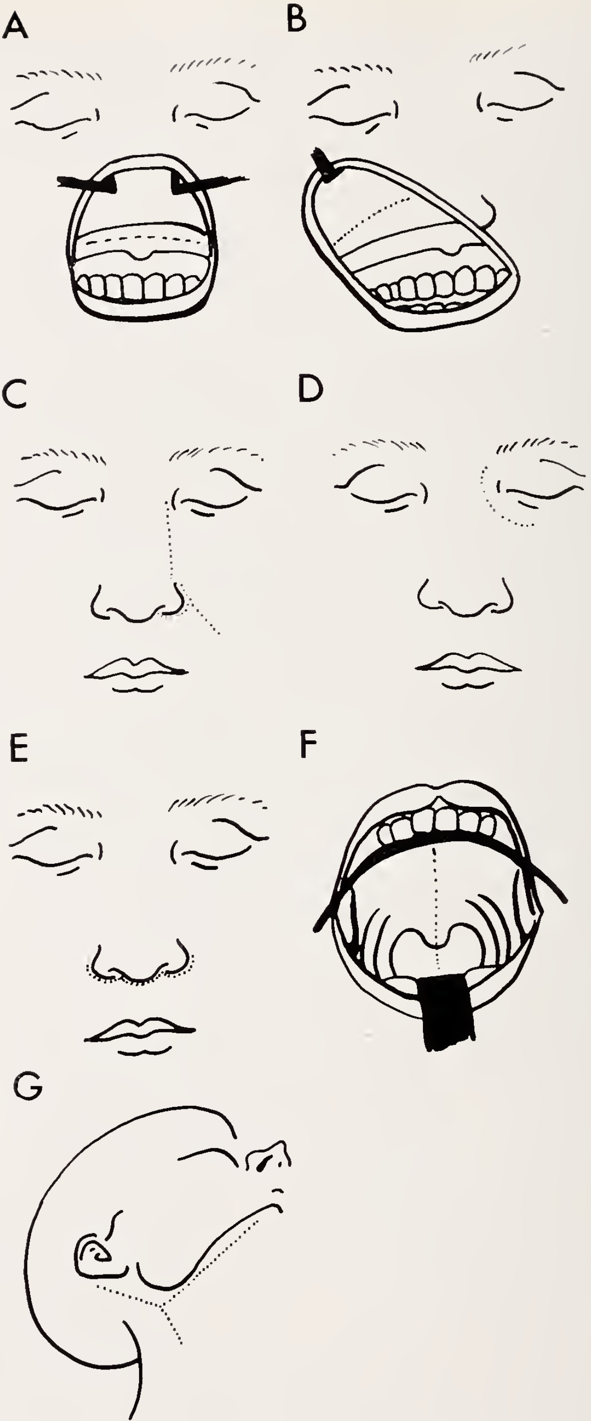

Fig. 1. Diagrams of selected initial incisions for transsphenoidal and transclival surgery. A = oronasal rhinoseptal; B = oro-antral nasal; C = transantral nasal; D = transethmoid sphenoidal; E = transnasal cavity; F = transoral to clivus; G = transcervical transclival.

host's tolerance of his own bacterial flora. It is also important to emphasize that all instruments used in the midline approach to the hypophysis have to be specifically modified in a bayonetted form. The small exposure under microscopic control leaves little room for manipulations at the surface. All instruments must have a minimum length of 13 cm after the proximal angulation.

The transoral approach to the low- and mid-clivus, as well as C1 and C2, permits a fast and easy exposure of the underlying skeletal structures. It is only necessary to carry the incision through the soft palate when one seeks to expose the clivus region. The mucosa of the pharynx can be incised along with the periosteum over the bodies of C1 and C2, but careful attention must be paid to avoid extending the vertical incision past the point of adequate visual exposure, since it is necessary to do a meticulous closure of these layers at the termination of the procedure (14). The transcervical transclival approach was developed by Stevenson (16) to avoid operating through a contaminated field. This has been the objection to the transoral approach by most neurosurgeons. A transverse incision is made in the submandibular region from just beyond the midline, extending posteriorly into the mastoid region. The head must be rotated to the opposite side and extended to allow entrance to the submandibular space. Originally, the vertical T was used for better mobilization of skin flaps and ease of cervical dissection. This has now been abandoned in favor of a completely transverse incision. Though the external and internal dissection can be accomplished by the same surgeon, the time of exposure and the entire procedure is shortened by the team approach, permitting a surgeon with experience in the deep cervical region to provide the exposure of the clivus. The neurosurgeon thus is fresh to concentrate his efforts on the exposure of the neural axis. The exposure obtained is usually 4½ cm in length and 2½ cm in width. Careful attention must be paid to stay medial to the atlantooccipital facet joints in removing the bone of the clivus to avoid injury to the twelfth nerve in the anterior condyloid canal and the neurovascular bundle entering the jugular foramen and foramen lacertum. Separation of the superior pharynx from the periosteum of the clivus is also an exacting procedure, because an opening in the pharynx would, therefore, convert the sterile procedure to a contaminated one, thereby losing the advantage for which so much excess dissection has been done. The head must be turned to a midline position before beginning the bony removal to avoid distortion of the anatomy. The need for a prosthetic replacement for the clivus bone is debatable, but certainly an attempt should be made to obtain a water-tight closure by use of a dural graft. In short, the disadvantages of the transoral approach are those of surgery through a contaminated region, and the necessity of meticulous closure of the mucoperiosteal layer. That of the transcervical method is the increased surgical dissection required, and the original obliquity of the exposure as well.

Selection among the transnasal, transoral, or transcervical approach to the clivus and upper cervical spine can usually be easily made (Fig. 2). The transnasal approach should be limited to the area of the clivus above the hard palate since the slope of the clivus below this point gives an oblique target away from the surgeon, a particular deterrent to surgery of the basilar artery. The

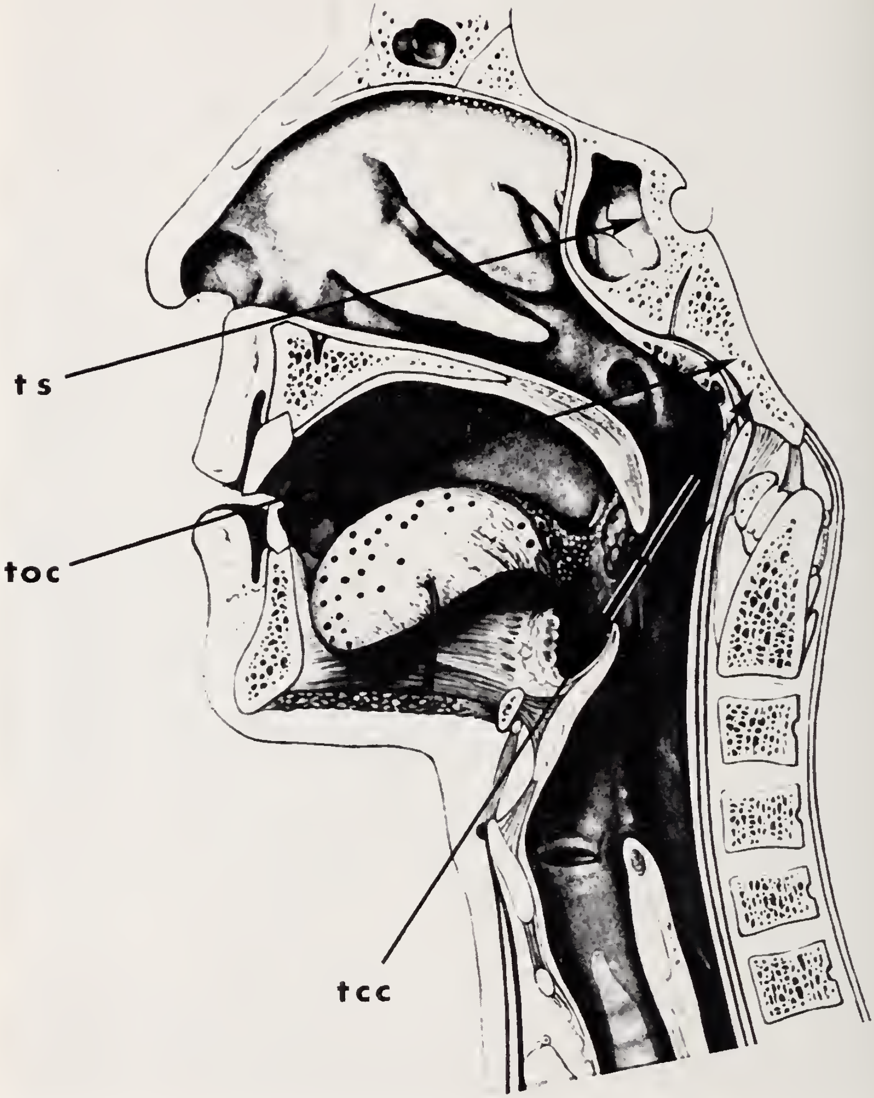

Fig. 2. Sagittal view of head and neck illustrating approaches to sella turcica and clivus. ts = transsphenoidal; toc = transoral to clivus; tcc = transcervical to clivus.

lower clivus can be equally exposed by the transoral or transcervical approaches, as can the upper cervical spine. If the exposure is to be limited to the C1–2 area, the transcervical approach is a simpler procedure than the full exposure of the clivus by this technique.

Surgical Lesions

The midline lesions which are under surgical consideration at the present, and some perhaps in the future, are listed in Table III.

CEREBROSPINAL FLUID FISTULAS

Cerebrospinal fluid fistulas in the anterior fossa or through the sella into the sphenoid sinus can be repaired from above, via craniotomy, or from below, via rhinoseptal (21) or transethmoid (7) approaches. Fistulas through the frontal sinus are repaired from above by both neurosurgeons and rhinologists by evacuating the mucosa, obliterating the dead space, and either repairing the dura or covering the sinus opening with a pericranial graft. Defects in the anterior fossa through the perpendicular plate of the ethmoid are customarily repaired from above by a unilateral or bilateral anterior fossa approach. The use of fascial grafts with or without tissue adhesive (COAPT) have been successful in our experience. Montgomery has led the rhinologists by popularizing his transethmoid approach with a septal mucosal flap applied to the undersurface of the perpendicular plate of the ethmoid to seal the leak. Fistulas that drain to the sphenoid sinus through the floor of the sella are difficult to seal from above without sacrificing the hypophysis (22). In the rare cases occurring with an intact pituitary gland, the better approach might be from below, using one of the transseptal or possibly transethmoidal techniques. In this repair, a septal mucosal flap would be used as a vascularized pedicle to seal the opening of the sellar floor. Fascia lata molded within the sella to replace a defective dura can frequently be helpful.

PITUITARY TUMORS

Transsphenoidal surgery for pituitary tumors was popularized by Cushing and Hirsch soon after the turn of the century. Even in that era of unsophisticated anesthesia, limitation of vision, and lack of antibiotic coverage, the results were certainly commendable. The mortality rates of Cushing and Hirsch were in the 3 to 4% range in over 300 cases of various neoplasms (23). Improvement in visual fields varied from 60 to 70%, comparable to modern craniotomy figures (24). Cushing deviated from the transsphenoidal route in the

TABLE III

Midline Lesions of Base of Skull and Upper Cervical Spine

Cerebrospinal fluid fistulas
Sphenoid sinus tumors
Pituitary tumors and remote processes requiring pituitary ablation
Craniopharyngiomas
Clivus chordomas and meningiomas
Basilar artery aneurysms and occlusions
Anterior communicating artery aneurysms
High cervical tumors, fractures, and dislocations

late 1920's because of its failure to provide satisfactory improvement in visual acuity in a significant number of cases. In Henderson's analysis of Cushing's statistics (23), he felt the transfrontal operation for pituitary tumors superior because of increased return of visual acuity and fields to complete normality. Of the total of 60 to 70% improving, 21% of the transfrontal cases reached normal, as against only 9% of transphenoidals. These statistics have to be taken with the knowledge that Cushing removed only $\frac{1}{3}$ to $\frac{2}{3}$ of the tumor in his usual transsphenoidal approach. Moreover, the patients in his later series of transfrontal operations were usually the recipients of postoperative radiotherapy.

During the 1920's, there was a phasing out of the transsphenoidal operation and a gradual transition to the intracranial procedure by most neurosurgeons. However, Hirsch (25), Hamlin (26), and Dott (23), among others, continued to use the transsphenoidal operation with excellent success. During the last 10 years, Guiot (18) has reevaluated this approach which, on the basis of a large number of cases, gives excellent functional results, with negligible morbidity and mortality. The introduction of such parasurgical adjuvants as radiofluoroscopic control by Guiot and the dissecting microscope by Hardy, represents a significant advance in this field. This microsurgical approach allows complete removal of the tumor in most instances, despite large suprasellar extensions. Hardy (5) advocates a transsphenoidal microsurgical approach to virtually all pituitary tumors that produce diminished visual acuity or visual field deficits. He reserves the transfrontal operation for those cases with lateral suprasellar extensions. Postoperative radiotherapy is not given in his cases in which complete removal was thought to have been accomplished. Instead, they are followed by plain x-rays to see the position of his postoperative clip on the sellar dura or tumor capsule. In the past year, Nicola (6) has successfully performed complete removals of hypophyseal tumors with large suprasellar extentions by a similar transsphenoidal approach. This microscopic transsphenoidal approach also enables the surgeon to possibly preserve the pituitary gland while removing a small, distinct tumor, or especially a microadenoma (Hardy, J.: Transsphenoidal Microsurgery, read at the Microneurosurgical Course, The Mount Sinai School of Medicine, New York, N. Y. 10029, May 2, 1969). In Hardy's large series treated by complete surgical removal, there have been no mortalities attributed to the procedure. Other neurosurgeons, notably Svien, have switched to the transsphenoidal approach in order to lower their mortality rates in patients with an excessively large sella turcica and, therefore, a large inferior extension of the tumor. It would appear that prior to giving radiotherapy to sellar masses with visual defects, transsphenoidal biopsy should be considered for diagnosis, possible treatment, and avoidance of radiotherapy to nonresponsive lesions.

Ray's selection of the transfrontal operation over the transsphenoidal procedure is based on his incomparable clinical results in several hundred cases with negligible operative mortality (24). His success is laudable, but certainly does not detract from transsphenoidal surgery. Visual improvement occurred in 70% of Ray's operated pituitary tumors, a figure which closely parallels that of the early results of Cushing and Hirsch. In our modest experience with the transfrontal microsurgical approach to pituitary tumors, we have been impressed with the ease of apparent complete removals without mortality or significant morbidity.

PITUITARY ABLATION

Pituitary ablation, usually for patients with metastatic carcinoma of the breast and diabetic retinopathy, can be easily accomplished with a negligible morbidity and mortality by the transsphenoidal microsurgical approach (4, 7, 20, 27). Hamburger, and later Hardy, have removed the pituitary in block by the extracapsular approach in which the pituitary is mobilized and its stalk sectioned low. It would seem that the total removal of the pituitary gland is essential, since piecemeal removal, leaving very small fragments of pituitary tissue, may leave the patient with significant pituitary hormone secretion, thus obviating an adequate operative procedure.

Bergland's (28) objection to transsphenoidal pituitary surgery, on the basis of the anatomic variance of the pituitary gland and adjacent structures, is somewhat biased. To decrease difficulties relating to these structures, under the direct vision of the microscope one can note variations of the anatomy of the carotid arteries, cavernous sinuses, and pituitary stalk.

CRANIOPHARYNGIOMAS

The management of craniopharyngiomas continues to be controversial even in neurosurgical circles. In the past, the standard therapy of craniopharyngiomas has been cyst evacuation, and/or partial removal of the neoplasm, followed by postoperative radiation therapy. Excellent results by this method of treatment have been claimed by Kramer and McKissock (29) in a series of 26 patients, both adults and children. After craniotomy, their patients received radiotherapy with a dose range of up to 6000 r's in 40 to 45 days in children, to over 7000 r's in 50 days in adults. Of ten patients treated by this planned surgical radiotherapeutic approach, one adult and six children were alive and well 13½ to 15 years after treatment, without evidence of recurrence or brain damage. In a later series (30), four out of six children and seven out of ten adults were alive and well 6 months to 8 years after treatment. On the other side of the coin, that of radical surgery, stood the recently deceased Donald Matson (31). He had advised total excision of the tumor without postoperative radiation therapy. In his experience with 57 children with craniopharyngioma, 44 were the recipients of total, and 11 of subtotal, excision of the tumor. There was a postoperative mortality in five cases, which were all secondary operations. Forty-four patients in the series remained alive to attend school and lead relatively normal lives, with follow-up periods extending to 20 years. Matson has stressed the importance of attempted radical excision at the first operative procedure, thus avoiding adhesions secondary to irradiation and previous craniotomy. The recent use of the surgical microscope has helped achieve total removal of craniopharyngiomas that may be deeply imbedded in the sella or attached to the hypothalamus and brain stem. We have been impressed by the necessity of magnified vision while stripping the capsule of the tumor from the neurovascular structures at the base of the brain.

Hardy has widened the use of his transsphenoidal approach to total removal of craniopharyngiomas without mortality in a limited number of cases (Oral Communication, January 1969). On occasion, he has also identified and preserved the normal pituitary gland during complete excision of the neoplasm. This approach would appear to be an excellent one for craniopharyngiomas, either for simple cyst evacuation, or radical removal (32). Further sophistication of this procedure in the future may lead to milder postoperative courses with less need for hormonal regulation, additionally avoiding the possible long term effects of massive radiation therapy.

CHORDOMAS

Chordomas located at the superior extremity of the notochord have always been difficult surgical problems (33). They may be located in the nasopharyngeal region, intrasphenoidal, or retroclival (34). The last two locations are classified as clivus chordomas. Guiot has characterized clivus chordomas in the following manner (17):

(1) *Intrasphenoidal Chordoma:* These patients characteristically present with a short history of diplopia, ophthalmoplegia, and trigeminal pain. Radiologically, there is extensive osteolysis of the sphenoid bone and petrous tips. Guiot has used an anterior rhinoseptal approach to remove these tumors in a benign and efficacious manner. However, in his two cases, despite adequate decompression and subtotal removal, recurrences appeared after 1 year in one case, and after 4 months in the other. This has been true in the experience of other surgeons attacking this type of chordoma (34, 35). The tumor is usually soft and suckable, with variable vascularity. We have recently encountered a huge intrasphenoidal and retroclival chordoma of this type in a 65-year-old male (Fig. 3).

Case Reports

Case 1. The patient was referred for surgery after the development of bilateral amblyopia and total ophthalmoplegia with a hemiparesis of varying laterality. A radical removal of this fairly soft and partially suckable tumor was accomplished using the midline transnasal cavity approach. Utilizing microscopy, the tumor was removed from its attachments between both the carotid and basilar arteries. At the conclusion of the procedure, most of the brain stem was in view since the tumor had grown and replaced the dura from the sphenoid to the odontoid process. There were traces of tumor visualized extending posteriorly around the brain stem in a circumferential manner, perhaps some strands extending superiorly and laterally into the sylvian fissure. A muscle pack was placed in the defect, followed by a tailored piece of fascia lata, and then the redundant mucosa of the bony septum and its sphenoid continuation was sutured in the midline to reinforce the defect. The patient's postoperative course was satisfying in that vision returned in both eyes by the first week. There was a continuous improvement in his general neurologic status, as well as a significant regression of his ophthalmoplegia. The patient continues to improve, approximately 6 months postoperatively. His only significant disability at present is a right sixth nerve

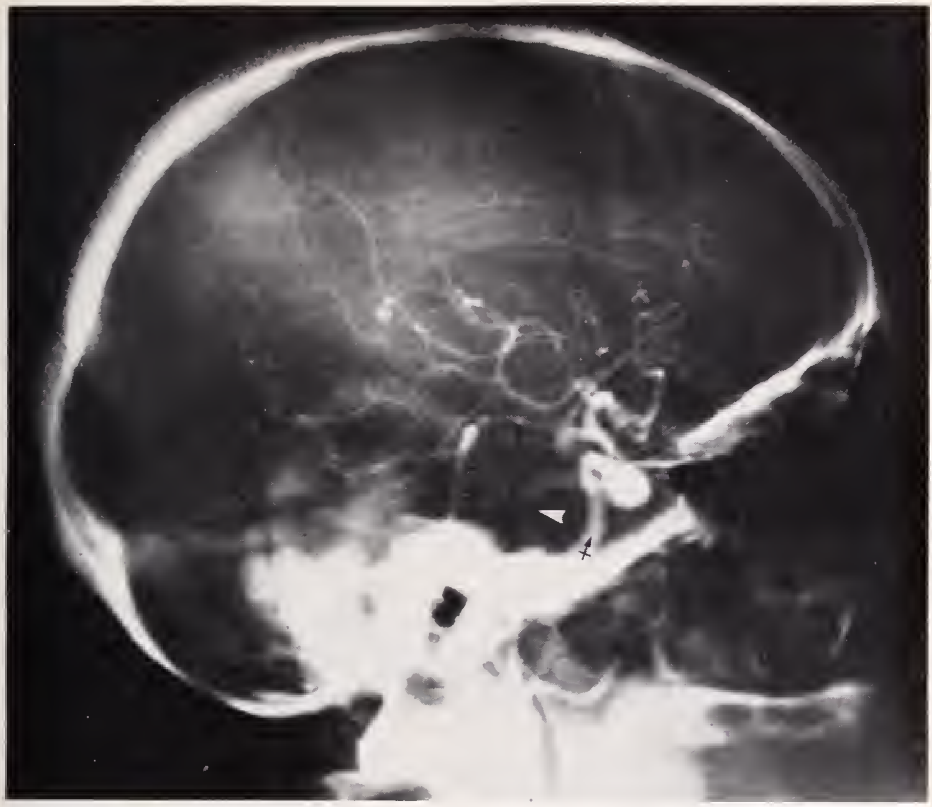

Fig. 3. Right brachial arteriogram, lateral view. Large intrasphenoidal and retroclival mass. Vertical arrow = markedly elevated and stretched posterior communicating artery; double arrow = posteriorly displaced and stretched basilar artery; horizontal white arrow head = posteriorly displaced and elevated left internal carotid artery (cavernous portion); crossed arrow = anteriorly and inferiorly displaced right internal carotid artery.

paresis. While it would seem that a complete extirpation of this lesion is beyond our present surgical abilities, an adequate decompressive palliation with low morbidity and mortality is quite possible.

Case 2. In another patient seen at an earlier stage (Fig. 4), the same operative approach was used, but the tumor was still entirely extradural and caused its vascular distortion by stretching of the dura. In this case it appeared that total removal with a clear bony margin was achieved. Nevertheless, only further observations will determine the possibility of actual cure by this method.

(2) *Retroclival Calcified Chordoma:* These patients usually have a long standing history with a paucity of neurologic signs. Despite the surgical problems associated with the dissection of fibrous and calcified tissue, they have a better prognosis with subtotal removal because of slow growth. When this tumor is located high and extends laterally to the parasellar region, the subtemporal route is probably the best approach with sectioning of the tentorium (33, 35–37). For the medially placed retroclival tumors, the rhinoseptal route seems to be quite competitive, and perhaps preferable, to the suboccipital or subtemporal approaches. Other midline approaches to retroclival chordomas have been the

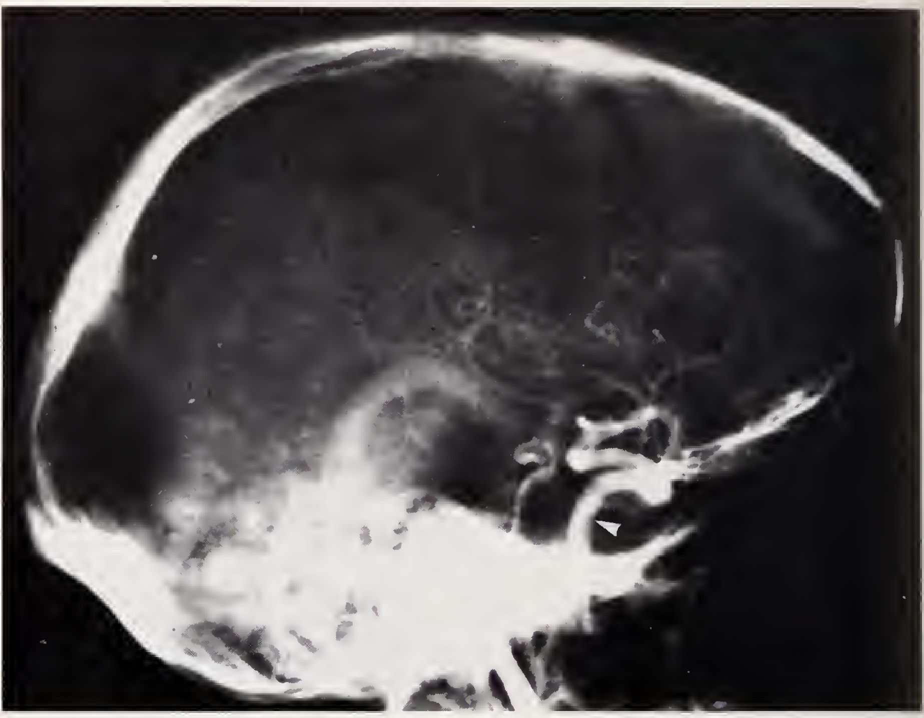

Fig. 4. Right brachial arteriogram, lateral view. Destruction of sella turcica and superior clivus by epidural chordoma. White arrow head = right internal carotid artery displaced posteriorly and elevated. Basilar artery displaced posteriorly.

transoral approach by Nissen and Klinger (38), the transcervical approach by Stevenson, and the transpalatal pharyngeal approach by Scoville.

MENINGIOMAS OF THE CLIVUS

Meningiomas of the clivus have been considered by Castellano and Ruggiero in a review of Olivocrona's cases to be inoperable by the standard suboccipital or subtemporal approaches (39). Attempts at total removal by the transoral approach by Mullan (15) were unsuccessful. However, partial removal and decompressive palliation seemed quite possible by this method. The meningiomas of the clivus frequently extend inferiorly to be classified with foramen magnum tumors, or sometimes extend laterally and superiorly to become posterior fossa, or even middle fossa tumors. A combined approach in planned stages seems to be a possible answer to the problem. The use of transclival, suboccipital, or subtemporal approaches may permit the total removal of this neoplasm when the clinical status warrants.

BASILAR ARTERY

Most aneurysms of the basilar artery can be successfully operated upon by the subtemporal route with section of the tentorium (40–43). However, aneurysms of the lower third of the basilar artery and basilar-vertebral junction are difficult to reach by this technique. They have been successfully attacked by Fox (44) and French (45), using the transcervical transclival approach. Yasargil, using the transoral approach to the midclivus, has had significant experience with difficult midbasilar aneurysms (Yasargil, G.: Microsurgery of Intracranial Aneurysms, read at the Microneurosurgical Course, The Mount Sinai School of Medicine, New York, N. Y. 10029, April 30, 1969). We have performed cadaver exposures of the basilar bifurcation, using Hardy's transsphenoidal approach, which may prove useful in attacking aneurysms imbedded in the interpeduncular cistern.

Recent experience in microvascular surgery has given us the technical ability to do endarterectomies of the basilar artery through a transclival approach. Reichman has successfully performed anastomoses between the lingual artery and basilar artery in dogs (Reichman, O. H.: Experimental Lingual-Basilar Microanastomosis, read before The American Association of Neurological Surgeons, Cleveland, Ohio, April 1969). Perhaps in the future this will be a way of increasing the blood supply to the brain stem through the basilar artery. Presently, we lack adequate criteria for performing basilar endarterectomies. The procedure would not appear to be indicated in acute basilar occlusion with serious and progressive brain stem signs. Patients with intermittent brain stem dysfunction and radiologic evidence of basilar occlusion or severe narrowing may be candidates for reestablishment of the basilar blood flow by endarterectomy.

ANTERIOR COMMUNICATING ARTERY ANEURYSMS

Marked improvement in the morbidity and mortality of anterior communicating artery aneurysms has been obtained since the routine use of the dissecting microscope (46, 47) (Yasargil, G., and Kurtze, T.: Personal Oral Communication, April 1969). To achieve these results, one must operate at a time when bleeding has stopped, the neurologic examination is markedly improved or normal, and there is a lack of evidence of arterial spasm on angiography. In our earliest cadaver exposures of the sella turcica and upper clivus via the transsphenoidal route, we mistakenly removed bone anterior to the sella turcica. Through this opening in the tuberculum sella and part of the planum sphenoidale, we exposed the optic chiasm and anterior communicating artery after incising the dura. With gentle retraction of the anterior chiasm, the anterior communicating artery and small portions of the proximal and distal anterior cerebral arteries and their perforating vessels came into view (Fig. 5). These are normally located slightly anterior and superior to the chiasm. With the use of elongated microalligator forceps, a ligature was secured, trapping the anterior communicating artery. Further development of long bayonetted instruments has allowed the placement of a clip on the anterior communicating artery. This transsphenoidal exposure could be useful in an anterior communicating aneurysm where bleeding continues and craniotomy must be deferred. Much experience must be gained in surgery of anterior communicating aneurysms

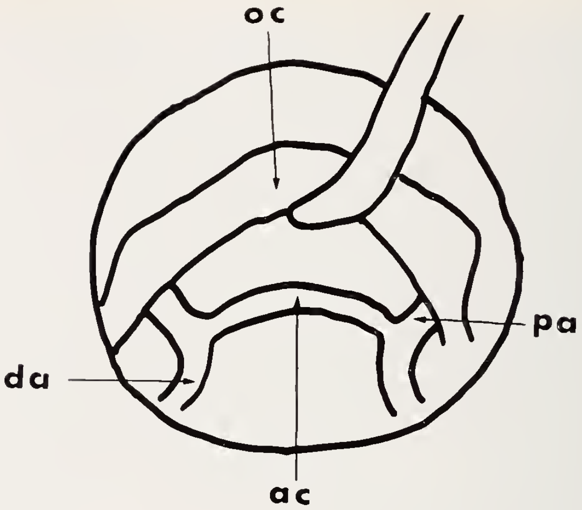

FIG. 5. Diagram of exposure of midline after dura of tuberculum sella and posterior aspect of planum sphenoidale is opened. Retractor lifting chiasm. oc = optic chiasm; ac = anterior communicating artery; da = distal left anterior cerebral artery; pa = proximal right anterior cerebral artery.

on patients classed as grade four by this method before it can be suggested as a useful surgical technique for optimum candidates. Marked anatomical variations severely limit animal surgery as a means of gaining further experience with this method. Prefixed optic chiasm certainly would be a deterrant to this procedure. However, Schaeffer (48), in his dissection of 125 cadavers, and Ray, with his extensive experience in hypophysectomy, have noted that the chiasm is prefixed in only 5 percent of all people.

UPPER CERVICAL SPINE

The midline approach to the upper cervical spine has been useful in selective instances for tumors of the bodies of C1–2, fusions of C1 and C2, and odontoid resections for cervical medullary compression. The transoral approach to the vertebral bodies has been used by Southwick and Robinson (13), Fang and Ong (14), as well as Scoville (12). Resection of tuberculous involved bone and replacement by iliac graft has been successful. The above surgeons have also used this approach for reduction and anterior fusion of unreducible fracture dislocations, rather than doing a standard posterior fusion after decompression.

A posterior graft must bridge the gap between the occipital bone and the spinous processes and posterior arches of the second and third cervical vertebrae for proper fusion. This is frequently cumbersome and, if successful, results in poor mobility of the occiput and upper cervical region.

Anterior fusion of C1–2 has been most frequently accomplished by wedging iliac bone (after partial odontoid resection) into the gaps between the lateral masses of the atlas and the articular processes of the axis (14). Successful anterior fusion at this level allows for nearly normal cervical mobility, while also preserving the occipital-atlas joints.

Summary and Conclusions

The use of the dissecting microscope plus televised radiofluoroscopic control has markedly enhanced the value of older and proven surgical approaches to the base of the skull. These technical advances have also led to the development of newer procedures, typified by the transcervical transclival approach to the brain stem.

Development of the surgical approaches has been traced in a chronological manner, stressing regional anatomy. Refinements and adaptations have been discussed along with suggestions regarding the distinctions upon which the selection of available surgical procedures may be made.

The following management of selected midline lesions is recommended:

(1) Cerebrospinal fluid fistulas through the ethmoid or sphenoid sinuses may be repaired transsphenoidally using one of several comparable approaches.

(2) Pituitary tumors can be totally removed transsphenoidally if there is some sellar enlargement and a medial rather than a significant lateral suprasellar extension.

(3) Pituitary ablation for carcinoma of the breast and diabetic retinopathy can be made total by the extracapsular removal which the transsphenoidal operation makes feasible.

(4) Total removal of craniopharyngiomas by the transsphenoidal technique may still allow preservation of the pituitary gland.

(5) Chordomas located intrasphenoidally or retroclivally have been successfully removed using the appropriate rhinoseptal or transclival procedure.

(6) Clivus meningiomas may prove resectable using the combination of transclival, suboccipital, or subtemporal approaches.

(7) Basilar artery aneurysms have been obliterated using the transcervical, transclival, and transoral techniques. Microvascular surgery of the basilar artery may be forthcoming.

(8) Anterior communicating artery aneurysm mortality has been reduced by microsurgical attacks using the bifrontal or unilateral subfrontal approaches. A method of the transsphenoidal exposure and trapping of the aneurysm has been suggested.

(9) Upper cervical spine disorders have proved amenable to midline anterior repair.

Most significant new tools lead the way to major advances in technique. The use of the surgical stereoscopic microscope and image intensifier radiofluorographic control promises a real revolution in the approach to the surgery of lesions of the base of the skull.

Generic and Trade Names of Drugs

COAPT—*isobutyl 2-cyanoacrylate monomer* (Ethicon, Inc., Sumerville, New Jersey).

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The Development of Pancreatic Secretory Insufficiency in a Patient with Recurrent Pancreatitis and Type V Hyperlipoproteinemia

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Recurrent pancreatitis associated with hyperlipoproteinemia is well known (1–6). Whether or not pancreatic secretory insufficiency develops after many years of such attacks has been only rarely recorded. Gross and Comfort (1) reported that no sequellae were encountered in two of their three patients with chronic relapsing pancreatitis and hyperlipemia. A third patient had diabetes mellitus. These authors do not mention how long they had followed their patients. Klatskin and Gordon (2) also found no sequellae in their patient with multiple attacks of pancreatitis and hyperlipemia studied for 5 years. A secretin test was attempted but a normal enzyme determination was found. No volume or bicarbonate studies were done. Adding to the difficulty in studying this problem is the fact that recent investigations (7, 8) have also shown that glucose intolerance is a cardinal feature of certain types of hyperlipoproteinemia, types IV and V, thereby making it impossible to use abnormalities in carbohydrate metabolism as an indication of pancreatic insufficiency.

We have had the opportunity of following a patient with primary hyperlipoproteinemia and relapsing pancreatitis symptomatic for over 10 years. This patient, who has been the subject of other reports (5, 9), has also been followed with serial secretin tests. More recently, she has been typed by the method of Fredrickson and Lees (7).

Case Report

The patient was first studied at The Mount Sinai Hospital in November 1955, when blood drawn during her fourth pregnancy was found to be lipemic (Table I). She was asymptomatic at this time, never having had abdominal pain or xanthomata. She was admitted in February 1956, while 8 months pregnant, with severe midline epigastric pain, nausea, and vomiting. Her white blood count was 10,200/mm^3, with a shift to the left. Serum amylase determinations were 204 U on admission, decreasing to 79 U before discharge. The patient responded to nasogastric suction, bed rest, anticholinergics, and intravenous fluids. Postpartum x-rays showed a normal gall bladder and upper gastrointestinal series. Serum lipid determinations initially showed cholesterol 498 mg%, phospholipids 660 mg%, and triglycerides 3,186 mg%. A 3 hour glucose tolerance test was normal (Table III). In addition, her first secretin test was normal with a volume of 3.5 cc/Kg, a bicarbonate secretion of 109 mEq/L, and her

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TABLE I

Serum Lipid Determinations

Dates	Cholesterol (mg%)	Phospholipids (mg%)	Triglycerides (mg%)	
11/55	498	660	3,186	No symptoms
2/56	240	343	1,373	Symptoms of abdominal pain
12/65	260	—	1,800	No symptoms
2/66	320	—	1,734	Symptoms of abdominal pain
11/66	320	500	1,600	No symptoms

TABLE II

Secretin Tests (12)

Dates	Volume (ml/Kg)	Bicarbonate (mEq/L)	Amylase (U/Kg)
2/56	3.5	109	15.1
11/63	3.2	102	21.4
2/66	1.8	75	7.8

* Lower limit normal values: volume = 2.0 ml/Kg; bicarbonate = 90 mEq/L; amylase = 6 U/Kg.

From Dreiling, D. A., and Janowitz, H. D.: The Measurement of Pancreatic Secretory Function, in *Ciba Symposium on the Exocrine Pancreas: Normal and Abnormal Functions*, de Reuck, A. V. S., and Cameron, P., eds., J & A Churchill, London 1962, p 225.

TABLE III

Glucose Tolerance Tests

Dates	FPS (mg%)	½ Hr (mg%)	1 Hr (mg%)	2 Hr (mg%)	3 Hr (mg%)
2/56	98	160	145	77	82
2/66	114	178	180	143	102
11/66	96	98	122	136	86

enzymes were 15.1 U/Kg. She was discharged and apparently did well until February 1957, when she was admitted again for severe midline epigastric pain, nausea, and vomiting. The white blood count was 9,700/mm^3, with a shift to the left. Serum amylase was 116 U, decreasing to 35 U prior to discharge. Again, treatment consisted of nasogastric suction, intravenous fluids, and anticholinergics.

After discharge from the hospital, the patient continued to have episodes of midline epigastric pain unrelated to foods, and she remained hyperlipemic. Because an oral cholecystogram showed several calculi, she was admitted for surgery, and had a cholecystectomy in May 1957 for cholelithiasis and chronic cholecystitis. The status of the pancreas was not mentioned in the operative note. Between 1957 and 1963, although attacks of abdominal pain, fever, nausea, and vomiting persisted, she did not seek medical attention. In September 1963 she presented with the above symptoms. Serum amylase was 57 U, and bilirubin was 0.6 mg%. Other acute phase studies were also normal. An intravenous cholangiogram was normal. In November 1963 she again was admitted for fever, pain, nausea, and vomiting. No diagnosis was made, and she was discharged. Amylase was 57 U, bilirubin 0.9 mg%, and the white blood count was 6,900/mm³. A second secretin test was done which was normal (Table II). Repeat intravenous cholangiogram and upper gastrointestinal series were normal. The patient's serum remained lipemic.

Symptoms persisted, and in February 1966, 8 days after her third secretin test, she was admitted to The Mount Sinai Hospital because of a 7 day history of fever, epigastric pain (relieved by leaning forward), and bilious vomiting. Physical examination was that of an apprehensive nonicteric 34-year-old female. Blood pressure was 126/70, pulse 80/minute, respirations 16/minute, and temperature 101°F. The abdomen was quiet, with guarding and midline tenderness. Rectal examination was normal. Her stool was brown without occult blood. The white blood count was 11,700/mm³, with a shift to the left. Erythrocyte sedimentation rate was 60 mm/hour. Urinalysis was normal. Her serum remained lipemic; the amylase was 356 U on admission, and 86 U on discharge. Again, no xanthomata were observed. All other laboratory tests were within normal limits. Treatment consisted of intravenous fluids, antibiotics, nasogastric suction, and anticholinergics. She responded to this regimen, and was discharged. The patient continued to have symptoms following a normal diet without alcohol, and was readmitted in November 1966 because of the same symptoms.

This patient has been moderately obese during her life, does not drink alcoholic beverages, and eats a standard American diet, including fats and carbohydrates. She denies diarrhea or weight loss.

Classification

During the past year we have attempted to classify this patient according to the phenotyping system for hyperlipoproteinemia developed by Fredrickson and Lees (7, 8). Prior to the day of study, this patient had been following her usual diet without restriction. After a 14 hour fast there was marked lipemia, the upper layer of which creamed on standing. Lipoprotein electrophoresis (Fig. 1) revealed a type V pattern with both chylomicrons and pre-beta lipoproteins being present. There was streaming of the pre-beta lipoprotein fraction on the electrophoresis. Polyvinylpyrrolidone separation (10) had only 18% of the triglycerides recoverable in the top layer (alimentary), and postheparin lipolytic activity (11) was normal (0.38 mEq/ml/minute; normal = 0.25 – 0.50).

The above pattern is most consistent with type V. The inconsistent features are the normal glucose tolerance test found when the patient was first discovered, and the fact that of the family members so far tested, her father, age 54, and one daughter, age 16, are normal. One daughter, age 10, may be abnormal, having the presence of excess pre-beta lipoproteins on the lipoprotein electrophoresis. Fredrickson, Levy, and Lees (8) report abnormal glucose

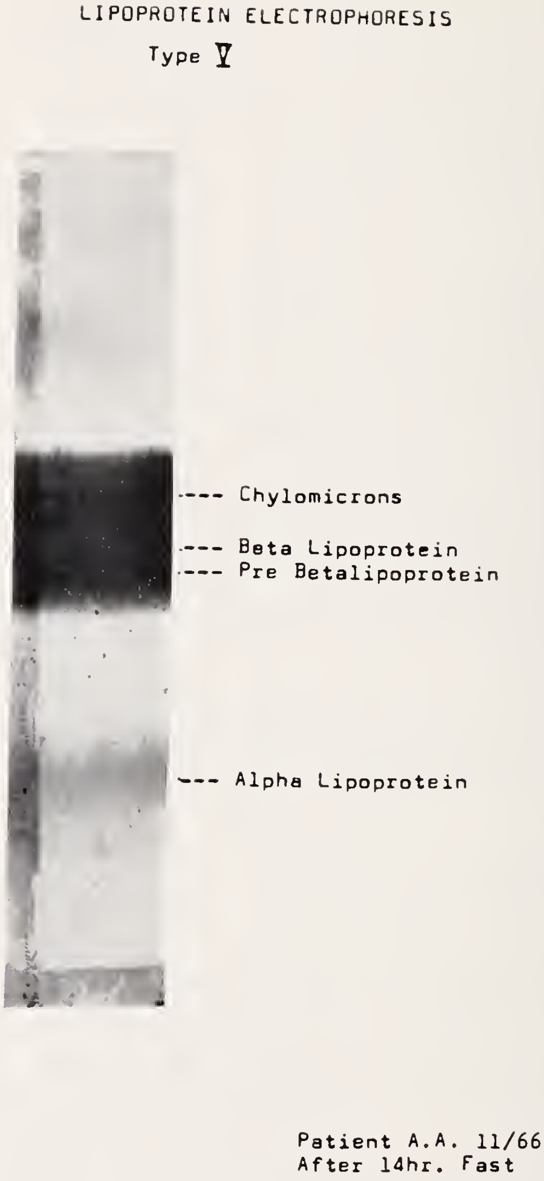
LIPOPROTEIN ELECTROPHORESIS
Type V
Chylomicrons
Beta Lipoprotein
Pre Betalipoprotein
Alpha Lipoprotein
Patient A.A. 11/66
After 14hr. Fast

Fig. 1. Lipoprotein electrophoretic pattern of patient A.A. after 14 hour fast. There is marked excess in both the chylomicron (exogenous) and pre-beta lipoprotein (endogenous) fractions. Streaming of the chylomicron fraction is present.

tolerance in almost all of their patients and family members, and hyperlipoproteinemia in all parents of their type V patients so far studied.

Comment and Summary

The above case report is one of a female with recurrent pancreatitis associated with primary hyperlipoproteinemia (type V). She was followed at our hospital for over 10 years, during which time she had multiple bouts of pancreatitis. Initially, both secretin studies and glucose tolerance tests were normal (Tables II and III). However, over the years, associated with multiple attacks of pancreatitis, enough pancreatic function has been destroyed so that both secretin studies and glucose tolerance became abnormal. This appears to be the first time that definite evidence of the development of pancreatic secretory insufficiency has been documented in association with the recurrent pancreatitis of essential hyperlipoproteinemia (type V—Frederickson).

Acknowledgment

The lipoprotein electrophoretic studies were kindly performed for us by Dr. Robert S. Lees, Rockefeller University, New York.

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Autoerythrocyte Sensitization Reproducible by Both Autologous Red Cells and Heterologous DNA

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Autoerythrocyte sensitization is a syndrome characterized by crops of painful ecchymoses. In the syndrome, as originally described by Gardner and Diamond (1), the typical lesions could be reproduced by the intracutaneous injection of autologous red blood cells. Ratnoff and Agle (2) reported 27 cases and extensively reviewed the literature. They renamed this disorder "Psychogenic Purpura" because the occurrence of this syndrome appeared to be confined to adult women with multiple somatic complaints and relatively uniform psychiatric features. In 9 of their 27 patients, the lesions could not be reproduced by the injection of autologous red blood cells. Therefore, these authors did not regard reactivity to autologous red blood cells as necessary for diagnosis.

Four cases have been recorded in the literature in which painful hemorrhagic cutaneous lesions similar to those present in autoerythrocyte sensitization were present, and which could be reproduced by intracutaneous injections of desoxyribonucleic acid (DNA) (3–6). No psychiatric data are available on these four patients.

We have recently seen a patient who presented with the typical syndrome of autoerythrocyte sensitization, as described by Ratnoff and Agle (2), including recurrent painful ecchymoses and a severe psychiatric disorder. Her lesions could be reproduced by the subcutaneous injection of both autologous red blood cells and heterologous DNA. This would suggest that the syndrome of autoerythrocyte sensitization and DNA autosensitivity may be related.

Case Report

This was the first Mount Sinai admission of an 18-year-old white female with the chief complaint of painful ecchymoses on the legs of 3 months' duration.

She had been in fair health until October 1968, when she developed an otitis media and lymphadenopathy. Two weeks later she noted numbness in her calves and swelling of both legs, accompanied by the abrupt onset of dime-sized, bluish red, tender swellings on the anterior tibial surfaces. She was hospitalized in a local hospital, where she was found to have a fever of 101–102°F and painful ecchymotic lesions of both legs. She also complained of arthralgias of her knees and elbows. There was no history of bleeding tendency, easy bruisability, hematuria, skin rash, abdominal pain, chest pain, or local trauma. She

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was treated with antibiotics, prednisone, and anticoagulants, and gradually improved over a period of 3 weeks. Upon discharge her lesions had cleared, and her medication had been discontinued. An extensive laboratory work-up was unrevealing. The day after discharge the lesions reappeared acutely, and they progressed to involve her legs, thighs, and arms. She was rehospitalized and treated with anticoagulants and prednisone, 50 mg/day, without response. Because of a suspected diagnosis of autoerythrocyte sensitization, she was started on cyproheptidine, 4 mg, four times a day, and the steroids and anticoagulants were discontinued. By the following day the pain had subsided, and within 3 days the lesions disappeared.

After discharge, she was advised to discontinue the cyproheptidine in preparation for skin testing. The following day painful lesions reappeared. She was admitted to The Mount Sinai Hospital for further study.

The patient stated that she could predict the appearance of lesions because of pain in the involved area prior to the appearance of the lesions. When in the cold, her fingertips would turn white. She had a history of episodes of weight fluctuation. She had had two gynecological operations: an ovarian cystectomy, and a uterine fixation. She had also undergone repair of a torn meniscal cartilage, appendectomy, and repair of a bilateral alternating exotropia. She was allergic to penicillin.

She had had a history of recurrent nightmares. The theme of these nightmares was a feeling of being pushed toward a casket in which she saw herself as a dead child. She had undergone psychotherapy at age 8 because of a refusal to go to school. At age 15 she was depressed and made two suicidal gestures: one by putting a razor to her neck, and another by taking an overdose of aspirin.

The patient had been under severe emotional stress prior to the initial appearance of her lesions. She and her husband had married 4 months earlier than originally planned, against the wishes of her in-laws. Her father-in-law became severely depressed and required electroshock therapy, and her mother underwent an exacerbation of a peptic ulcer.

Positive physical findings were limited to the skin. There were a number of warm, exquisitely tender, erythematous ecchymotic lesions on her left arm and both anterior tibial surfaces. The calves were tender to deep pressure. No objective arthritis or neurological abnormalities were found.

An extensive laboratory investigation was undertaken, and the following tests were all within normal limits: complete blood count; sedimentation rate; platelet count; urine analysis; blood urea nitrogen; fasting blood sugar; CO_2; chlorides; sodium; potassium; bilirubin; calcium; phosphorus; cholesterol; alkaline phosphatase; uric acid; lactic dehydrogenase; glutamic oxaloacetic transaminase; creatinine phosphokinase; bleeding and clotting times; prothrombin time; latex fixation; lupus cell preparation; antinuclear antibody; antiDNA antibody; direct and indirect Coombs' test; protein bound iodine; T_3 resin uptake, haptoglobin; cold agglutinins; protein electrophoresis; IgG; IgM; IgA; VDRL; antestreptolysin O titer; electrocardiogram; urinary creatine; creatinine; chest x-ray; and tuberculin skin test.

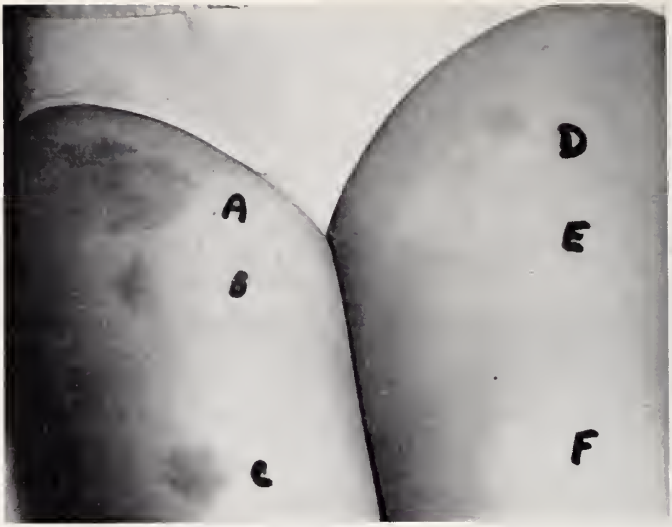

FIG. 1. A = Site where 1.0 mg of calf thymus DNA had been injected 1 day previously; B and C = sites where 0.1 cc of autologous whole blood had been injected 2 days before; and D, E, and F = control sites where 0.1 cc of saline had been injected.

When the lesions were at their height, the β_1C complement was 65 mg%; this rose to 100 mg% when the lesions remitted.

Skin and muscle biopsies were taken from the gastrocnemius and left tibialis anterior. The skin, muscles, and blood vessels were normal.

0.1 cc of the patient's whole blood was injected intracutaneously into the right thigh, and 0.1 cc of saline into the left thigh. Over the next hour the patient developed a warm, painful, erythematous, 8 × 10 cm area in the right thigh. The left thigh remained normal. After this lesion cleared, she was injected with 0.1 cc of a solution containing 1.0 mg of calf thymus DNA that had been sterilized by passage through a millipore filter. She had a marked reaction to the DNA injection with the appearance of an 8 × 12 cm warm, erythematous, ecchymotic area. The control area injected with saline remained normal (Fig. 1). The appearance of the DNA solution and saline were similar, and the patient did not know what had been injected at either site.

A psychiatric consultation described the patient to be a friendly and cooperative 18-year-old female with no thought disorder, but one who had been under severe emotional strain. The patient was the older of two sisters in a family where both parents were inhibited in the expression of anger. Her mother was described as being overprotective and avoiding confrontation. The psychiatrist thought that the patient had a true psychosomatic disorder. She was felt to be trapped between unconscious dependency needs and wish for maturity in an externally difficult situation.

Subsequent to discharge, the patient was maintained on cyproheptidine, with partial suppression of her lesions.

Discussion

The patient described here resembles the patients described by Ratnoff and Agle (2), both in regard to the cutaneous lesions, and the psychiatric disorder.

Of great interest was the ability to reproduce lesions with injection of either autologous red blood cells or heterologous DNA. No evidence of a connective tissue, hematological, or autoimmune disorder could be demonstrated to account for the patient's symptoms.

In view of these findings, it would seem likely that autoerythrocyte sensitization and autoDNA sensitization may be related entities, the etiology and pathogenesis of which are totally unknown. This would seem to agree with the view of Ratnoff and Agle (2) that both entities be called "Psychogenic Purpura," and that the diagnosis be made on the basis of typical clinical and psychiatric features, rather than on the basis of the exogenous agent capable of reproducing the typical lesions.

Of special interest in this case was the moderately depressed serum complement present during active ecchymoses, which later returned to normal with healing of the lesions. This point, as many others, requires further study.

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Hirschsprung's Disease in a Premature Infant

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Since the original description of aganglionic megacolon by Hirschsprung in 1888 (1), there have been no well documented instances in the literature of the occurrence of this entity in premature infants. We recently followed a premature female infant who manifested abdominal symptomatology in the neonatal period. Rectal biopsy confirmed the diagnosis of Hirschsprung's disease, and definitive surgery was performed.

Case Report

A 1,760 gram female infant was born to a 24-year-old Negress at 36 weeks gestation. The membranes were ruptured for an undetermined time prior to delivery. Labor was spontaneous, and delivery was uneventful. Physical examination of the infant at birth was normal. Abdominal distention was first noted at 2 days of age. The distention subsided after passage of meconium. The infant did well until 8 days of age, when there was the sudden onset of irritability, refusal of feedings, and marked abdominal distention. A plain x-ray film of the abdomen showed distended loops of small intestine, without distention of the large bowel. The baby was thought to be septic, and therapy with penicillin and neomycin was started after appropriate cultures were obtained. A Levin tube was passed, and intravenous fluid therapy was instituted. An enema was given, and a large formed stool was passed. Twenty-four hours later the distention was less. B. proteus was isolated from cultures of the throat, nasopharynx, and blood. By 11 days of age the baby was passing normal stools, and physical examination of the abdomen was unremarkable. Oral feedings were restarted. She continued well and was discharged at 6 weeks of age, weighing 2,730 grams. A barium enema done prior to discharge was normal.

The infant was readmitted to the hospital at 8 weeks of age because of increasing abdominal distention, vomiting, and diarrhea. Rectal examination was unremarkable. With gastric decompression and intravenous fluid therapy, the child improved over a 24 hour period. Two days later a barium enema showed narrowing of the distal segment of the rectum and dilated large bowel proximal to the stenotic area (Figs. 1A and 1B). A tentative diagnosis of Hirschsprung's disease was made, and the baby was taken to the operating room, where a transverse colostomy was performed. Biopsy specimens from the colostomy site showed many ganglion cells. No rectal biopsy was done. The postoperative course was complicated by a gastroenteritis, which responded to intravenous hydration and oral polymyxin. No bacterial pathogens were isolated on stool culture. The baby was discharged at 3 months of age, weighing 3,100 grams.

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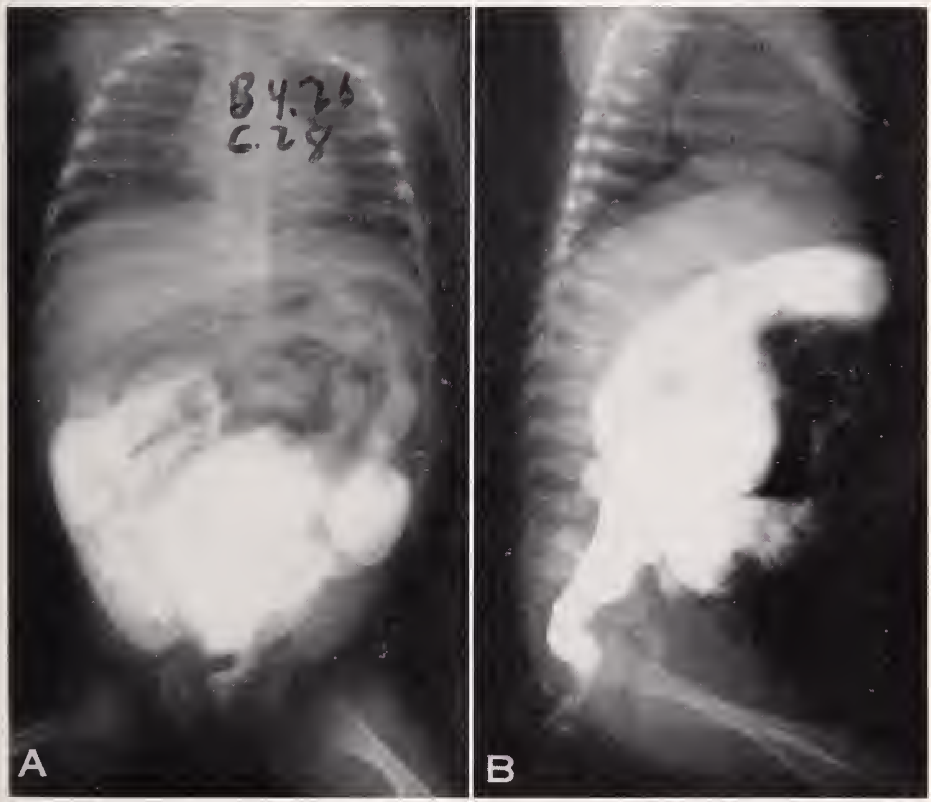
The child was followed in the outpatient clinic for the next 16 months, and did well. At 2 years of age she was readmitted for definitive surgical correction of her colonic lesion. Rectal biopsy at that time showed segments of smooth muscle of bowel wall with some small nerve trunks, but without ganglion cells. A Duhamel procedure was subsequently performed. Pathological specimens showed aganglionosis extending to the midsigmoid colon.

Comment

Although the clinical picture of Hirschsprung's disease in older children is well known, the manifestations of this disease in infants are often not widely appreciated. Symptoms almost always date back to the neonatal period. The greatest proportion of infants with aganglionosis present with intestinal obstruction, gastroenteritis, or chronic diarrhea with failure to thrive (2). The clinical picture may resemble sepsis, with abdominal distention, poor feeding, regurgitation, vomiting, and lethargy.

Failure to diagnose aganglionosis carries the risk of development of necrotiz-



FIGS. 1A and 1B. Posteroanterior and lateral roentgenograms taken immediately after a barium enema, showing dilatation of the large intestine above a relatively stenotic distal segment.

ing enterocolitis, a potentially lethal disease which may occur in 50 percent of these infants (3). Repeated attacks of abdominal distention, vomiting, and constipation may be a further consequence of undiagnosed Hirschsprung's disease (4).

Various theories have been proposed to explain the etiology of aganglionosis (5). One report (6) considers Hirschsprung's disease to be a developmental anomaly in which migration of neuroblasts along the alimentary tract ceases at various stages before the 12th week of intrauterine life. If this theory is correct, one might expect to find Hirschsprung's disease occurring more frequently in premature infants than has heretofore been the experience.

Despite the absence of case reports in the literature, it seems unlikely that Hirschsprung's disease selectively avoids premature infants. Yet if the latter be true, it would be very important to the understanding of the epidemiology of the disease. However, it is more logical to assume that the diagnosis of aganglionosis is frequently missed when it occurs in premature infants during the early weeks of life. This is particularly true, as our case demonstrates, if the infant concurrently has sepsis.

Conclusion

Hirschsprung's disease should be considered in any premature or full-term infant presenting with abdominal distention and a clinical picture of sepsis. Since sepsis is a common complication of prematurity, the diagnosis of aganglionosis is not usually considered. Barium enema and/or rectal biopsy should be done once the diagnosis is suspected, no matter how ill the infant appears. If the diagnosis is confirmed, colostomy should be performed immediately to avoid the feared complication of enterocolitis.

Acknowledgment

The authors wish to thank Dr. Lotte Strauss, Department of Pathology, Mount Sinai School of Medicine, for interpretation of the pathological specimens.

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Evaluation of Palliative Operations for Carcinoma of the Head of the Pancreas: A Ten Year Study

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Despite modern investigational methods, carcinoma of the head of the pancreas continues to be a major surgical problem. The incidence is listed as 0.3–0.9% of all autopsies, and 1–2% of all malignancies (1, 2). According to several large recent surveys, by the time patients are hospitalized, only about 20–30% of them are resectable (3–7). This study is concerned with the evaluation of various palliative operations for carcinoma of the head of the pancreas.

Material and Methods

The material consists of analysis of case records of 50 patients from the Bronx Veterans Administration Hospital during the 10 year period from 1957 to 1967. All were proved cases of carcinoma of the head of the pancreas. One was epidermoid carcinoma, and the remainder were adenocarcinoma. All were male patients. The youngest patient was 34 years old, the oldest was 87, and the mean age was 55 years. Abdominal pain was the most common symptom, and was experienced by 66% of the patients. Weight loss, jaundice, and anorexia were the next most prominent symptoms. Kern, Creutzfeldt, and Kummerle found painless jaundice in only 16% of the patients in their analysis of 150 patients with carcinoma of the head of the pancreas (8). Thus, progressive painless jaundice is not the most common symptom, as was believed in the past. Icterus was the most frequent physical finding, being present in 66% of the patients. Hepatomegaly was present in 40% of the patients.

All 50 patients had been operated upon (Table I). Ten patients underwent exploratory laparotomy, biopsy, and closure, and 40 patients had some type of bypass procedure. The overall operative mortality rate was 30%, and the mortality for bypass procedures only was 25%. Operative mortality is defined as death occurring within 30 days from the date of surgery. Four patients were lost to follow-up, 2, 4, 6, and 12 months after surgery. Patients who had exploratory laparotomy alone lived 2.2 months, and the average survival of patients with a bypass procedure was 5.5 months. One patient who underwent multiple bypass procedures lived for 5 years. Seven patients, 17.5%, had a biliary bypass procedure without a gastroenterostomy. These patients had a second operation, gastrojejunostomy, from within 1½ months to 2 years, for relief of upper gastrointestinal obstruction. Glantz and Ozeran report a high incidence of 50% of the patients developing subsequent upper gastrointestinal obstruction requiring reoperation (9).

Comparative data of the average survival in bypass procedures in months and mortality rate is listed in Table II. Our survival rate of 5.5 months is in

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accord with the other large series. In the analysis of termination of these patients, 58% died of progression of their disease, 10% had terminal cholangitis, 8% had massive upper gastrointestinal bleeding, and only 4% died of unrelated causes (Table III).

Significant x-ray findings after barium meal such as widened duodenal sweep, external compression of the duodenum, or anterior displacement of the pylorus were present in 56% of the patients. Splenoportogram was done on two patients, and in one was positive for hepatic metastasis; an osseous venogram was positive in one patient. Arner and Fernstrom (10), while performing splenoportogram on 66 patients, could demonstrate metastasis only in 9. They conclude that if a metastasis is less than 3 cm in diameter, it will not be visualized by splenoportogram.

Comments

It is clear from the above analysis that biliary bypass procedures have a definite role in the management of unresectable carcinoma of the head of the pancreas. Every patient explored, however, should have a thorough evaluation for resection. Criteria for pancreaticoduodenectomy include Kocherizing the duodenum, feeling for the retroperitioneal lymph nodes, dividing the gastroduodenal artery, and feeling the portal vein for any infiltration. If the nodes, portal vein, and superior mesenteric vein are free of disease, resection may be considered. If the case is inoperable, the patient should have a biliary bypass procedure and a gastroenterostomy at the same time. In performing the biliary bypass procedure, preference should be given to choledochal anastamosis rather than to using the gall bladder, if the duct is free of disease. Buckwalter, Lawton, and Tidrick have shown in a large study that patients in whom the common bile duct was used live twice as long (7.5 months) as the mean survival of patients in whom the gall bladder was used (4.0 months) (11). In our own series, three patients had to undergo revision of cholecystojejunostomy to choledochal anasto-

TABLE I

Procedure and Survival in Months

Type of Operation	No. of Patients	Average Survival in Months
Exploratory laparotomy: biopsy and closure	10	2.2
Cholecystojejunostomy (loop)	9	3.5
Cholecystojejunostomy (loop) and gastrojejunostomy	12	4.5
Roux-en-Y cholecystojejunostomy	5	3.6
Choledochoduodenostomy	1	0
Gastrojejunostomy	1	2.0
Roux-en-Y cholecystojejunostomy and gastrojejunostomy	2	3.0
Cholecystojejunostomy and jejunojejunostomy	3	3.5
Biliary bypass procedure and subsequent gastrojejunostomy for upper GI obstruction*	7	18.7

* One patient with multiple bypass procedures lived for 5 years.

TABLE II

Comparative Data: Bypass Procedures

Source of Material	No. of Patients	Average Survival in Months	Operative Mortality
A cooperative study, eight hospitals or Portland	248	5.4	18%
Bufkin et al, Tulane University	157	4.7	16%
Kern et al, University of Freiberg	74	4.5	21%
Datsenko and Botkin, Hospital, Moscow	152	6.0	—
V. A. Hospital, Bronx, N. Y.	40	5.5	25%

TABLE III

Mode of Termination

	No. of Patients	Percent
Progression of the disease	29	58
Ascending cholangitis, hepatic abscesses, and toxemia	5	10
Massive upper GI bleeding	4	8
Unrelated causes	2	4
Cause not known	6	12
Lost for follow-up	4	8

mosis because of obstruction of the cystic duct. Besides, common bile duct bypass relieved jaundice and itching more effectively than cholecystojejunostomy. Biliary bypass procedure is palliative, clears jaundice, and relieves pruritis and pain. There is subjective improvement and, most of all, the patients believe that they are getting cured (11, 12), a psychological benefit. Consideration should be given to addition of vagotomy to the palliative procedure, since 8% of the patients died of massive upper gastrointestinal hemorrhage.

Summary

Fifty patients had been operated upon for advanced carcinoma of the head of the pancreas during a 10 year period from 1957 to 1967. Forty patients underwent different biliary bypass procedures, with an average survival of 5.5 months. The mortality rate for these patients was 25%. A total of seven patients underwent subsequent gastroenterostomy for upper GI obstruction. Our experience indicates that better results can be obtained by simultaneous double bypass (choledochoenterostomy and gastrojejunostomy) as an initial procedure. Further benefit may be possible by the addition of vagisection to this palliative procedure.

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Advances in the Clinical and Laboratory Evaluation of Renovascular Hypertension*

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After Goldblatt et al (1) in 1934 described the production of hypertension in dogs by clamping the renal arteries, many attempts were made to extend the knowledge acquired from this phenomenon toward the solution of certain clinical problems. A wave of unilateral nephrectomies in various centers followed. These kidneys which were affected by a variety of conditions, chiefly unilateral pyelonephritis, were removed in the hope of curing the hypertension presumably produced by them. Analysis of the results of these operations by Smith et al (2) revealed that fewer than 20 percent were followed by "cure" of the hypertension. In this connection it must be remembered that blood pressure tends to fall after any operation, and "cure" of the hypertension must be defined as the persistence of normal blood pressure for 1 year or more after surgery.

This type of surgery for hypertension was largely abandoned until 1953, when Howard et al (3), in four cases, described changes in kidney function produced by unilateral obstruction of the renal artery, and "cure" of the hypertension by nephrectomy. Perera et al (4) reported similar experiences in a few cases, and Poutasse (5) reported a series of cases of "cure" of hypertension, largely after nephrectomy, in patients with renal arterial narrowing or occlusion. This was followed by a report of renal artery repair and/or bypass by DeBakey and Morris et al (6, 7) in a large series of cases in which the hypertension was said to be "cured."

The diagnostic advances that made all this possible were: (1) the description of the functional abnormalities in the affected kidney by Howard et al (3), and then by Stamey et al (8) and others, consisting of a specific degree of decreased sodium excretion and urine volume on the affected side; (2) the development of the rapid sequence intravenous pyelogram (9) and the radioactive renogram (10); and (3) the development of aortography via the translumbar (11), and later the femoral arterial route (12).

In the more purely clinical sphere, a distinctive systolic "bruit" was described in this condition, usually heard best in the epigastrium, and the onset of the hypertension was found to be fairly abrupt and in age groups both younger and older than those affected by essential hypertension (13). Several types of arterial obstruction were described clinically, radiologically, and pathologically, the most common being caused by an atherosclerotic plaque (14, 15). Another cause of obstruction was fibromuscular hyperplasia of the renal artery, particularly seen in young women and often bilateral (16). Obstruction due to

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TABLE I

Statistical Analysis of Studies of 23 Normotensive (N) and 36 Hypertensive (H) Subjects

Study	Group	95% Range	Mean	Difference between Means	S.E.d*	p**
Work of digital vasoconstriction (10^3 ergs)	N	0.30–3.1	1.7	—	—	—
	H	0.20–6.6	3.4	1.7	0.31	$<10^{-7}$

* S.E.d = standard error of the difference.
** p = probability that the difference is due to chance.

TABLE II

Reactivity to Infused L-Norepinephrine (NE) after Vasodilatation

	Work of Vasoconstriction (10^3 ergs) Mean ± S.D.	Rate of NE Infusion (μg/min) Mean ± S.D.	Work/μg/min (10^3 ergs/μg/min) Mean ± S.D.
Normotensive (16 patients)	0.86 ± 0.28	26 ± 8.0	0.031* ± 0.011
Hypertensive (16 patients)	2.4 ± 1.1	18 ± 8.5	0.16* ± 0.10

* Standard error of the difference, $\sigma_D = 0.026$; $p < 10^{-6}$.
From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

aneurysm, tumor, and thrombosis was rarer. Bilateral atherosclerotic obstruction with hypertension and uremia was also described (17).

Some sour notes, however, began to appear in this chorus of enthusiasm. It was discovered by pathologists that obstruction of the renal artery or arteries could be present in many cases without ever having produced hypertension (18, 19). Other observers reported control of the hypertension by antihypertensive drugs in many cases of hypertension associated with renovascular obstruction (16). The original reports (6, 7) of over 80 percent "cures" of hypertension after surgery were gradually modified until it became clear that only about 40 to 50 percent of the cases could be expected to be "cured" of their hypertension (20–22). The "improved" cases were always suspect, but at any rate had to be analyzed separately.

It was thus obvious that improvement in the results depended almost entirely on better selection of cases prior to surgery. The first problem was to determine whether the renal artery obstruction was "physiologically significant." Attempts to do this on radiological grounds, as well as on the basis of the criteria established by Howard et al (3) produced some improvement, but cases still appeared in which the hypertension was not cured by adequate surgical intervention despite their meeting the established criteria (22). In addition, the Howard and Stamey tests occasionally produced unfortunate urinary tract infections (22). Renal vein renin determinations also were helpful in predicting

TABLE III

Reactivity to Infused L-Norepinephrine (NE) after Vasodilatation in Raynaud's Disease

Patients	No.	Work of Vasoconstriction (10^3 ergs) Mean ± S.D.	Rate of NE Infusion (μg/min) Mean ± S. D	Work/μg/min (10^3 ergs/μg/min) Mean ± S.D.
Normotensive.........	16	0.86 ± 0.28	26 ± 8.0	0.031* ± 0.011
Raynaud's disease.....	13	0.85 ± 0.64	16.7 ± 8.8	0.044* ± 0.021

* Standard error of the difference, $\sigma_D = 0.0064$; $p < 10^{-1}$.

From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

TABLE IV

Reactivity to Infused L-Norepinephrine (NE) after Vasodilatation in 23 Subjects with Renal Hypertension

Diagnosis	Resting Brachial Blood Pressure	Work of Vasoconstriction (ergs/μg NE base/min)
Chronic glomerulonephritis	180/110	32
Acute glomerulonephritis	(Before recovery) 160/104	72
	(After recovery) 126/80	148
Chronic glomerulonephritis	160/114	66
Diabetes mellitus; renal artery occlusion	224/100	44
Polycystic kidneys	156/100	82
Polycystic kidneys	146/86	122
Acute glomerulonephritis	146/82	104
Right renal artery stenosis	208/108	44
Obstruction left common iliac and right renal artery	136/78	96
Fibromuscular hyperplasia right renal artery; underlying essential hypertension (?)	(Before nephrectomy) 180/110	258
	(After nephrectomy) 116/76	172
Chronic glomerulonephritis	160/96	106
Renal hypertension	152/90	56
Bilateral renovascular hypertension	208/100	92
Chronic glomerulonephritis	164/120	175
Chronic glomerulonephritis	160/118	110
Chronic glomerulonephritis	140/100	79
Lupus nephritis	130/100	70
Chronic glomerulonephritis	140/110	167
Renovascular hypertension	200/102	186
Renovascular hypertension	160/86	67
Myeloma nephritis	176/106	24
Renovascular hypertension	224/130	184
Fibromuscular hyperplasia renal arteries	230/126	120

95% Normal range = work of vasoconstriction (ergs/μg NE base/min) = 46 to 206.

TABLE V

Percent Change in Digital Vascular Reactivity to L-Norepinephrine (NE) Produced by Prednisone Administration (30 mg Daily for 3 Weeks) as Measured by Percent Change in Work of Vasoconstriction per μg NE Infused per Minute

	Mean % Change	±S.D.
Normotensive (10 subjects)	+168*	±108
Hypertensive (9 subjects)	−11*	±18

* Standard error of the difference, $\sigma_D = 37$; $p < 10^{-5}$.

From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

TABLE VI

Statistical Analysis of Digital Circulatory Studies in 15 Normotensive Patients and in 15 Patients with Essential Hypertension

	Phase	Before Aldosterone Mean ± S.D.	After Aldosterone Mean ± S.D.
Normotensive (15)			
Work of vasoconstriction per μg NE/min (10^3 ergs)	C	0.049* ± 0.010	0.058* ± 0.014
Change in work of vasoconstriction per μg NE/min (%)	C	—	+21*** ± 30
Essential hypertension (15)			
Work of vasoconstriction per μg NE/min (10^3 ergs)	C	0.131** ± 0.040	0.233** ± 0.071
Change in work of vasoconstriction per μg NE/min (%)	C	—	+90*** ± 61

C = after vasodilatation and infusion of L-norepinephrine (NE).

* Standard error of the difference = 0.0044; $p < 0.05$.

** Standard error of the difference = 0.021; $p < 10^{-6}$.

*** Standard error of the difference between hypertensive and normotensive patients = 17.5; $p < 10^{-5}$.

From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

TABLE VII

Percent Change in Digital Vascular Reactivity to L-Norepinephrine (NE) Produced by Chlorothiazide or its Congeners as Measured by Percent Change in Work of Vasoconstriction per μg NE Infused per Minute

	Mean % Change	±S.D.
Normotensive (16 patients)	+62*	±69.0
Hypertensive (17 patients)	−32*	±28.0

* Standard error of the difference, $\sigma_D = 19.0$; $p < 10^{-5}$.

From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

TABLE VIII

Effect of Spironolactone on the Digital Circulation in 16 Patients with Essential Hypertension

	Phase	Before Spironolactone Mean ± S.D.*	After Spironolactone Mean ± S.D.*	S.E.$_d$**	p***
Work of vasoconstriction (10^3 ergs)	B	2.8 ± 1.1	2.0 ± 1.4	0.47	$<10^{-1}$
	C	1.5 ± 0.79	0.90 ± 0.45	0.23	$<10^{-2}$
Rate of NE base infusion (µg/min)	C	4.3 ± 1.1	4.3 ± 0.77	0.33	>0.5
Work of vasoconstriction (ergs/µg NE base/min)	C	350 ± 131	209 ± 97	41.0	$<10^{-3}$

B = before vasodilatation.
C = after vasodilatation and infusion of L-norepinephrine (NE).
* S.D. = standard deviation.
** S.E.$_d$ = standard error of the difference.
*** p = probability that the difference is due to chance.
From Mendlowitz, et al: Amer Heart J, C. V. Mosby Co., St. Louis, Missouri 1968, 76:795.

TABLE IX

The Effect of Drugs on the Digital Circulation in Hypertension

	Phase	Before Mean ± S.D.*	After Mean ± S.D.*	S.E.$_d$**	p***
Work of vasoconstriction per µg NE per min (10^3 ergs), guanethidine (15 patients).......	C	0.163 ± 0.063	0.267 ± 0.10	0.031	$<10^{-3}$
Work of vasoconstriction per µg NE per min (10^3 ergs), alpha-methyl dopa (15 patients)....	C	0.148 ± 0.060	0.218 ± 0.071	0.024	$<10^{-2}$

C = after vasodilatation and infusion of L-norepinephrine (NE).
* S.D. = standard deviation.
** S.E.$_d$ = standard error of the difference.
*** probability that the difference is due to chance.
From Mendlowitz, et al: Geriatrics, Lancet Publications, Inc., Minneapolis, Minnesota 1965, 20:797.

good results after surgery, especially if there were two or three times the amount of renin coming from the affected, as against the unaffected side (23, 24). Renin assays, however, are complex and fraught with possibilities for error. The older methods end with determination of the blood pressure change in an animal produced by angiotensin II, which is elaborated after incubation of the plasma for a specific period of time to permit the renin to produce angiotensin I, and converting enzyme to produce angiotensin II (25, 26). Newer methods depend on the same incubation, but determine the amount of angiotensin II or I pro-

TABLE X

Reactivity to Infused Tyramine (T) after Vasodilatation

	Work of Vasoconstriction (10^3 ergs) Mean ± S.D.	Rate of T Infusion (mg/min) Mean ± S.D.	Work/mg/min (ergs) Mean ± S.D.
Normotensive (15 patients)	0.78 ± 0.33	2.6 ± 1.1	306* ± 90
Hypertensive (15 patients)	0.86 ± 0.42	1.1 ± 0.50	839* ± 489

* Standard error of the difference, $\sigma_D = 128$; $p < 10^{-4}$.

From Mendlowitz, et al: Dis Chest, American College of Chest Physicians, Chicago, Illinois 1967, 52:709.

TABLE XI

Reactivity to Infused Angiotensin II (AT) after Vasodilatation

	Work of Vasoconstriction (10^3 ergs) Mean ± S.D.	Rate of AT Infusion (μg/min) Mean ± S.D.	Work/μg/min (10^3 ergs/μg/min) Mean ± S.D.
Normotensive (15 patients)	0.62 ± 0.3	1.9 ± 0.7	0.35* ± 0.13
Hypertensive (15 patients)	1.5 ± 0.9	1.1 ± 0.3	1.4* ± 0.68

* Standard error of the difference, $\sigma_D = 0.026$; $p < 10^{-6}$.

From Mendlowitz, et al: Circulation, American Heart Association, Inc., New York, N. Y. 1962, 25:231.

TABLE XII

Half-Time of Disappearance of H^3-NE from Plasma beginning 3 Hours after Cessation of Infusion

	No.	Mean	Range
Normotensive	5	240*	215–253
Hypertensive	5	183*	123–210

* $p < 0.05$.

From data presented by Gitlow, et al: J Clin Invest, American Society for Clinical Investigation, Rockefeller Press, New York, N. Y. 1964, 43:2009.

duced by radioimmune techniques (27–29). The surgeons attempted to improve their results by measuring change in pressure (30) and even flow rates (31) across the obstruction, establishing specific criteria as to whether or not to proceed with vascular repair or nephrectomy. This, however, could leave the patient with an unnecessary surgical intervention. Even with these techniques surgical failures still occur, although the success rate now is higher than it was.

The most important cause of failure is the association of renovascular hyper-

TABLE XIII

Apparent L-Norepinephrine Distribution Index

	No.	Mean ± S.D.*
Normotensive	25	45.2 ± 15.7
Untreated essential hypertensive	25	14.8 ± 6.9
Pheochromocytoma	3	619.0 ± 322.4
Renal hypertensive	3	45.5 ± 16.8

* S.D. = standard deviation.

From Wolf, et al: in manuscript.

tension with essential hypertension. Epidemiological studies have indicated that about one in ten persons in this country either has, or will have, essential hypertension (32). Furthermore, essential hypertension acclerates atherosclerosis (33), and hence the development of renovascular hypertension due to atherosclerotic plaques. One may therefore probably expect at least one out of every five cases of renovascular hypertension to have both essential and renovascular mechanisms operative. Regardless of all criteria for establishing the "physiological significance" of the renovascular disease, "cure" of the hypertension can not be expected through surgery in any patient who has both diseases. To insure such a "cure," it is necessary not only to establish the diagnosis of renovascular hypertension by renal vein renin determination and other criteria, but also to exclude the diagnosis of essential hypertension.

Our laboratory has been largely concerned with establishing tests for the positive identification of essential hypertension. By measuring pressure and flow in the digital circulation both before and after vasodilatation and converting the data into radius equivalents, and from these values and the pressures, estimating the work of vasoconstriction, we were able to demonstrate increased vasoconstriction in both essential hypertension (34) and Raynaud's disease (35). These methods took into account differences in venous pressure, critical closing pressure, and intravascular distending pressure, thus making it possible to compare disparate groups of patients properly (Table I). Since grossly increased vasoconstriction in the digit can only be due to increased sympathetic neural discharge, increased response to such discharge, or to both factors, we decided to test the responsiveness of the blood vessels by infusing L-norepinephrine (NE) intravenously after blocking neural discharge by indirect heat and chemical sympathetic ganglion blockade. There was an average fourfold increase in reactivity in the patients with essential hypertension (36), and little if any increase in such reactivity in Raynaud's disease (35) (Tables II and III). We therefore considered the vasoconstriction to be due largely to increased vascular reactivity in essential hypertension, and to increased neural discharge in Raynaud's disease (35). We also accumulated a large series of cases of renal hypertension of various types, including acute and chronic glomerulonephritis and renovascular hypertension, in which vascular reactivity was found to be normal (37) (Table IV). This seemed to exclude the possi-

TABLE XIV

After Intravenous Injection of 8 μg of D,L-β-H^3-Norepinephrine

Sex	Age	Blood Pressure (mm Hg)	% Excreted in 24 Hours
Hypertensives			
F	25	148/110	79.83
M	26	140/100	68.17
M	35	140/110	75.48
F	36	158/100	79.84
M	40	140/100	76.83
M	41	165/105	91.00
F	50	160/100	80.35
M	52	200/120	81.90
M	56	160/100	75.25
M	56	200/102	71.38
F	56	170/100	86.30
M	58	150/110	67.95
M	61	160/120	76.89
M	62	150/120	80.37
F	65	180/110	76.38
			Average = 77.86
			Range = 67.95–91.00
			S.D. = 5.95
Normotensives			
F	28	110/70	59.33
F	30	116/76	38.05
M	31	130/86	59.16
M	34	120/80	62.50
M	36	124/80	53.24
M	38	150/68	43.11
M	40	150/70	47.50
M	47	130/80	51.95
F	50	130/70	43.20
F	52	158/80	39.24
M	55	130/80	62.96
M	62	130/84	60.05
F	62	150/80	59.38
F	63	140/74	51.10
M	65	130/80	54.35
			Average = 52.34
			Range = 38.05–62.96
			S.D. = 8.15

Comparison of averages: D.F. = 28; t = 9.46; p = 0.01.

Parenchymatous renal and renovascular hypertensives were again well within the normal range.

From Gitlow, et al.: J Lab Clin Med, C. V. Mosby Company, St. Louis, Missouri 1969, 73:129.

bility that the increased reactivity in essential hypertension was caused by structural factors such as smooth muscle hypertrophy or intimal changes, since such changes were also present in both Raynaud's disease and in renal or renovascular hypertension.

We then turned to the possibility that the findings in essential hypertension were of biochemical origin. There was other physiological evidence which supported this hypothesis. Glucocorticosteroids can increase vascular reactivity in normotensive subjects, but not in patients with essential hypertension within 3 weeks (38) (Table V). Aldosterone and salt increased reactivity in normotensive subjects very little, but produced a marked increase in patients with essential hypertension in only 3 days (39) (Table VI). Sodium depletion by either a thiazide drug (40) (Table VII), or by spironolactone (41) (Table VIII), decreased reactivity in patients with esential hypertension in 2 weeks. Drugs which depleted NE stores such as guanethidine (42) (Table IX), or methyl dopa (42) (Table IX), decreased blood pressure but increased reactivity in essential hypertension within a few weeks. Tyramine, which released endogenous NE, also produced more response in the essential hypertensive than in the normotensive subject (43) (Table X), and the same effect was observed with angiotensin II (44), which is now known to have an effect on sympathetic nerves (45) in addition to its other effects (Table XI).

We next turned to investigation of possible biochemical abnormalities in essential hypertension. The attractive hypothesis that there was a defect in enzymatic breakdown of NE (46) in the periphery was proved false by failure to affect reactivity by administering iproniazid in essential hypertension, a drug which inhibits monamine oxidase (47). In addition, output of metabolites of NE in essential hypertension was fluctuant, but usually normal (48), indicating normal activity of the major degrading enzyme described by Armstrong et al (49) and by Axelrod (50), namely catechol-O-methyl transferase. Angiotensin II labeled with I^{131} disappeared more slowly from the plasma in essential hypertension (51), but we were not sure that this was specific, and finally attributed this phenomenon to increased angiotensin II stores, particularly in the more advanced form of the disease. *In vitro* degradation of angiotensin II was also studied, and the changes found in essential hypertension (52) were confirmed by some authors (53, 54), but not by others (54, 55), and we were not sure of their specificity.

The first positive result of our efforts, which became a lead, was the discovery by Gitlow et al (56) that the plasma disappearance rate of tritiated NE was more rapid in essential hypertension than normal (Table XII). Difference could not be found, however, by both Gitlow et al (57) and others (58), when urinary specific activity changes of NE and its metabolites were studied after infusion of H^3-NE. Profound changes in urinary specific activity were found, however, after administration of a variety of drugs which affect catecholamine metabolism such as reserpine, guanethidine, and pargyline (58, 59).

The first practical biochemical test for essential hypertension was then described in our laboratory by Wolf et al (60). Wolf gave 40 μg of D,L-β-H^3-NE

(1 mCi) intravenously in a single injection, and then measured the specific activity of its metabolite, normetanephrine, in the subsequently collected 24 hour urine. The apparent norepinephrine secretion rate (ANESR) or the NE distribution index (NEDI) was calculated by dividing the amount of H^3-NE given by the specific activity of urinary normetanephrine (NM). In essential hypertension (20 cases) the values were all below normal, largely because of increased tritiated NM in the urine (Table XIII). In renovascular hypertension and parenchymatous renal hypertension the results were normal.

A second biochemical test, also specific for essential hypertension, was then described in our laboratory by Gitlow et al (61). This consisted of injecting a single intravenous dose of 8 μg of H^3-NE (200 μCi), and then simply counting tritium in the subsequently collected 24 hour urine. This was called the tritiated NE uptake (TNEU) test. More than 65 percent of the injected radioactivity was excreted in the urine by the patients with essential hypertension in 24 hours (15 cases), and less than 65 percent by normotensive subjects (15 cases). Parenchymatous renal and renovascular hypertensives were again well within the normal range (Table XIV) (Fig. 1).

We now consider these two biochemical tests and the digital vascular reactivity test to be specific for essential hypertension. Certain precautions, however, are necessary. All patients should be tested for pheochromocytoma first, by measuring the output of urinary metabolites such as metanephrines or vanillylmandelic acid (VMA), before the tests for essential hypertension are

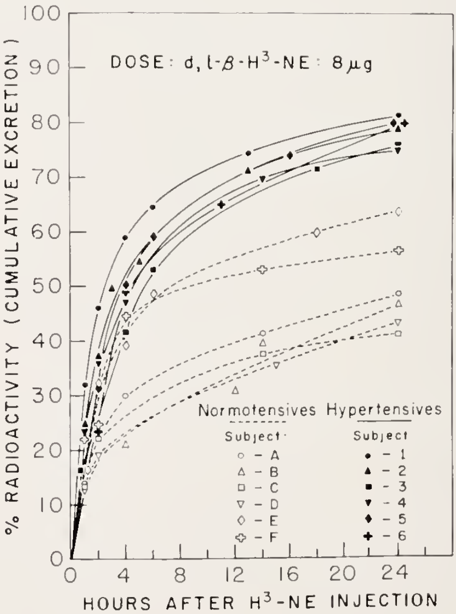

Fig. 1. Comparison of tritium excretion of six hypertensive patients and six normal subjects at 8 μg dose of D,L-β-H^3-norepinephrine (reproduced from Gitlow, et al: Journal of Laboratory and Clinical Medicine. C. V. Mosby Company, St. Louis, Missouri 1969, 73:129).

carried out (48, 49, 62–64). The digital vascular reactivity test is contraindicated if the hypertension is due to pheochromocytoma. The ANESR is much above normal (60) and the TNEU in the essential hypertensive range (61) when the patient's hypertension is caused by this tumor. Steroid hypertension should also be excluded by the usual clinical and laboratory investigations since it may affect the testing, and all drugs should be discontinued for about 2 weeks before testing. The patients also should not be uremic. Under these conditions, we have found the tests to be normal in uncomplicated renovascular and parenchymatous renal hypertension, as well as in early labile hypertension presumably due to anxiety only (65, 66). In early labile essential hypertension, however, the tests are positive. The high incidence of essential hypertension, and therefore of mixed cases particularly of essential plus renovascular hypertension, must be kept in mind. If the patient has bilateral renal arterial obstruction with hypertension and some degree of azotemia (17), and the aim of surgery is amelioration of renal function rather than "cure" of hypertension, then tests for essential hypertension are unnecessary. If the objective, however, is "cure" of the hypertension, we consider negative tests for essential hypertension together with high titer of renal vein renin on the side of renovascular obstruction to be strong indications for surgery, and thus far have had no failures in a very small series. We are trying to develop a larger series of cases based on these criteria.

Summary

(1) The history of surgical intervention in renovascular hypertension is reviewed.

(2) The results have been improved by demonstrating increased renal vein renin production on the side of the lesion.

(3) Three tests have been described which are specific for essential hypertension: digital vascular norepinephrine (NE) reactivity; apparent NE secretion rate; and tritiated NE uptake.

(4) It is believed that the surgical results will be improved further in the presence of normal tests for essential hypertension, and this has proved to be true thus far in a few cases. A larger series is being developed.

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Intranasal Surgery for Cerebrospinal Fluid Rhinorrhea

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The patient with cerebrospinal fluid rhinorrhea is under the constant threat of meningitis, brain abscess, and ultimate death. The pathogenic organism primarily responsible for these complications is the pneumococcus, the organism primarily responsible for otogenic meningitis. The fact that the pneumococcus can cause meningitis in these situations is certainly a subject worthy of investigation.

The method of treatment used to stop the leak, as well as the correct time to institute the treatment, are controversial issues. It is our belief that, in selected cases, the intranasal procedure described by Vrabec and Hallberg (1) should be utilized as soon as the diagnosis of cerebrospinal fluid rhinorrhea is made and the patient is properly evaluated.

History

The first documented case of cerebrospinal fluid rhinorrhea was described by Miller in 1826. Reported cases appear in the literature as early as 1676, in which Willis reported a patient who had a clear fluid draining from his nose. However, it was not until the early 1920's when Grant (2), and later Dandy (3), proposed surgical treatment for cerebrospinal fluid rhinorrhea. They approached the site of the leak through a craniotomy, and closed it by suturing the defect in the dura, or by suturing a transplant of fascia lata in place. The intranasal approach to the treatment of these cases was limited to cauterization prior to 1948. This technique did not have much success. In 1948, Dohlman described the use of a septal and middle turbinate flap to cover the defect in the cribriform plate. He approached this area through an incision used for an external ethmoidectomy. He then removed the bony wall of the olfactory groove to reach the dura (4). In 1964, Vrabec and Hallberg described an intranasal approach utilizing an advancement flap of middle turbinate mucosa to cover the defect in the cribriform plate after it had been denuded of its mucosa (1).

The procedure involves exposing the cribriform plate area intranasally. A submucous resection of the nasal septum is usually necessary to create good visualization. The cribriform plate is then denuded of its mucosa. At this time the fistula is usually seen, as well as the defect in the cribriform plate. The middle turbinate mucosa is then elevated from the medial surface down to the

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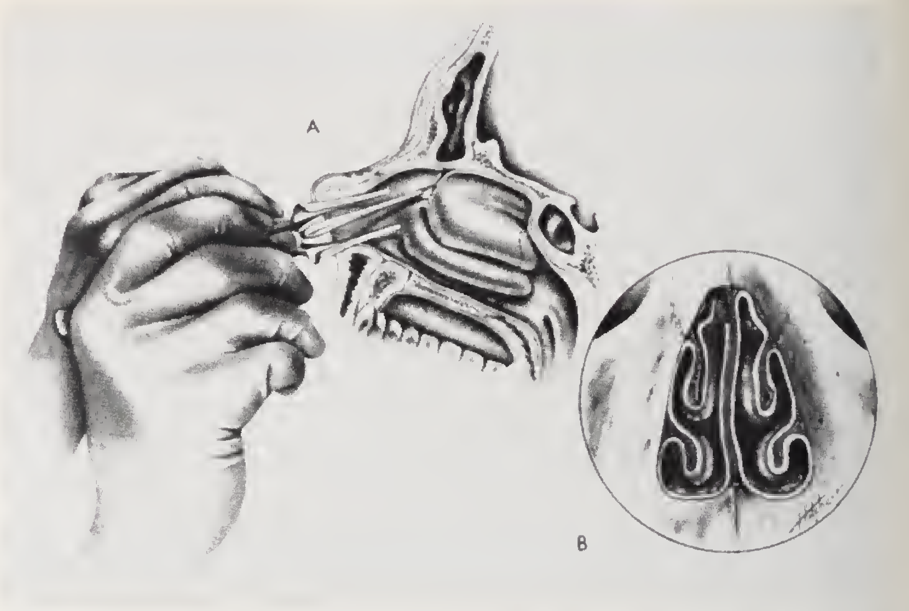

FIG. 1. A. Incisions for elevation of mucosal flap from medial surface of the turbinate bone. The anterior incision is made along the most anterior edge of the turbinate; B. frontal view, showing mucosal flap separated from the turbinate bone (courtesy of Dr. O. E. Hallberg).

free edge, where it is reflected laterally. The bone of the middle turbinate is removed, and the mucosa then utilized as an advancement flap to cover the cribriform plate area. This procedure is detailed in the diagrams and descriptions (Figs. 1–3), and was used in the following two cases.

Case Reports

Case 1. T.F., (MSH #306934), a 44-year-old Caucasian widow, was admitted to The Mount Sinai Hospital Otolaryngology Service on June 27, 1966, with a chief complaint of a clear fluid discharge from the left side of her nose for 16 years. This occurred especially on bending forward, and had followed a rhinoplasty. The interval between the rhinoplasty and the discharge was not remembered by the patient. On May 13, 1966 the patient developed a pneumococcal meningitis, and was treated for 19 days in another hospital. It was this illness that prompted a reevaluation of the nasal discharge. Upon examination, the clear discharge could be visualized as emanating medial to the insertion of the left middle turbinate, and could be easily produced by the patient by bending forward. Examination of the fluid revealed a sugar level of 58 mg%. Skull and sinus x-rays were normal. The spinal fluid pressure was 110 mm. The determination of sugar and protein performed upon the spinal fluid and simultaneously obtained nasal discharge were identical (sugar 61 mg%, total protein 18 mg%). There were no white blood cells or red blood cells in the spinal fluid, and no growth appeared on culture. A neurosurgical consultation was obtained. A pneumoencephalogram was recommended by the neurosurgical consultant, but the patient refused to undergo any neuroradiologic or neurosurgical procedure.

In view of the long history and the negative neurological physical examination, it was decided to attempt a repair of the fistula by the intranasal route. On July 7, 1966 the area

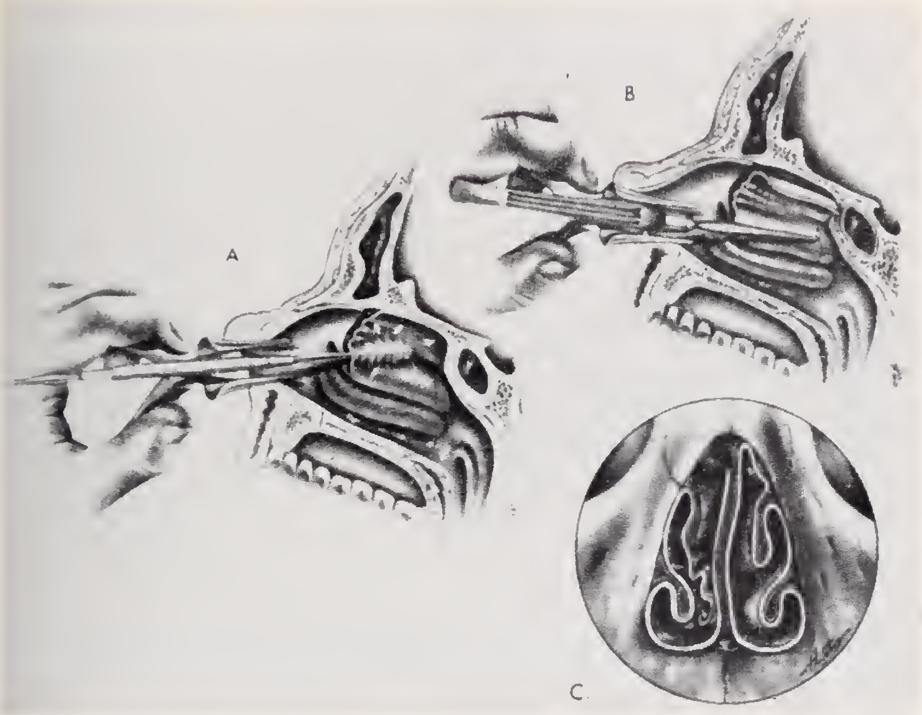

FIG. 2. A. and B. Elevation of mucosa from bony portion of turbinate; C. frontal view, showing mucosa separated from medial aspect of the turbinate, but remaining attached to the reflection of mucosa adherent to the lateral aspect of the bony turbinate (courtesy of Dr. O. E. Hallberg).

of the left cribriform plate was explored intranasally under local anaesthesia, with the patient in a sitting position. A submucous resection of the nasal septum was performed to gain better visualization. A fistulous tract was noted in the midportion of the cribriform plate, with a 3 mm defect in the bone of the plate surrounding the tract. No emanation of fluid was present, and none could be produced by jugular compression or by having the patient bend forward. The tract was then cut, the left cribriform plate denuded of mucosa, and an advancement flap created from the mucosa of the left middle turbinate, as described by Vrabec and Hallberg (1). This was done by first incising the anterior rim of turbinate along its vertical axis, and then elevating the mucosa from first the medial, and then the lateral side of the turbinate. The bone of the turbinate was then removed with biting forceps, taking care not to remove bone from the superior portion of the turbinate where it adjoins the cribriform plate. The removal of the bone allowed the mucosa to be advanced over the cribriform area. The flap was held in place by half inch vaseline gauze packing, applied over absorbable gelatin sponge (Gelfoam) in the cribriform area. The nose was first unpacked on the 7th postoperative day, leaving only a small amount of packing adherent to the flap. All packing was removed by the 11th postoperative day. The patient has had no recurrence of leak at this time (34 months after surgery).

Case 2. R.B., (MSH #120458). The second case is that of a 19-year-old Caucasian male who entered The Mount Sinai Hospital in July of 1967 with a chief complaint of right sided clear nasal discharge. This had occurred after a sleighing accident in 1959 in which a metallic foreign body penetrated the medial border of his left eyebrow, causing loss of

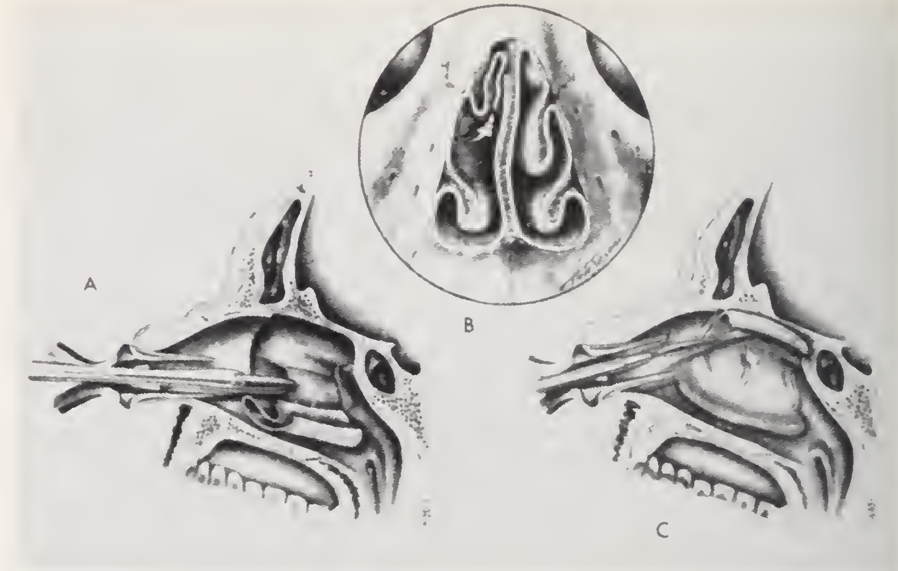

FIG. 3. A. After elevation of the mucosa from its lateral surface, the middle turbinate bone is removed with biting forceps; B. and C. the mucosal flap is then raised into position against the lateral nasal wall and cribriform region. The flap is held in place with oxidized cellulose gauze packing (courtesy of Dr. O. E. Hallberg).

vision in his right eye and a right sided cerebrospinal fluid rhinorrhea. An intracranial repair in 1959 demonstrated a defect in the cribriform plate, and a muscle plug and bone wax were used to seal this defect. The repair eventually broke down, and he was again operated on in 1963 by an intracranial approach; this time fascia were used to seal the defect. He had a total of six episodes of meningitis, the last four episodes occurring the year prior to surgery. He was admitted to the Neurosurgery Service. A pneumoencephalogram demonstrated a normal ventricular system. Intranasal pantopaque did not enter the intracranial cavity. A RISA study showed that the material remained in the subarachnoid space, not entering the nose, but this was felt to be due to adhesions. Intranasal examination revealed a septal deviation to the right which hid the cribriform area, but there was a clear fluid discharge provoked upon bending the head forward.

On August 3, 1967 an intranasal repair was performed using the technique described above. A submucous resection was necessary to adequately expose the cribriform plate, which was found to contain a fistulous tract in its midportion. This tract passed through a longitudinal fracture of the plate. There was a defect of the plate posteriorly, with the nasal mucosa retracted into the area of the defect. The mucosa on the nasal side of the cribriform plate was denuded, and a middle turbinate advancement flap created and advanced over the cribriform plate area. No leak was demonstrated at surgery. The patient was unpacked on the 11th postoperative day, and was discharged on August 18, 1967. There has been no recurrence of meningitis at the time of the writing of this paper (24 months after the surgery).

Comment

In using the intracranial approach, it is necessary to cut the olfactory nerves for adequate exposure; the intranasal procedure always avoids this. It is performed under local anaesthesia, and the morbidity and mortality are negligible;

in fact, we feel it is comparable in risk to repairing a deviated septum. The patient could be discharged from the hospital as early as the first postoperative day, which is never the case when using the intracranial approach. There are certainly cases of cerebrospinal fluid rhinorrhea on record that have persisted many years without developing complications, even without the use of antibiotics. There are also cases reported of patients who died shortly after the diagnosis was made. There is no doubt of the ever present danger of retrograde infection when a fistula between the nasal cavity and the subarachnoid space exists. A cough, a sneeze, or just the act of swallowing may reverse the pressure differential and cause retrograde flow. If an infection in the upper respiratory tract or in a sinus exists, the chance of infection is even greater.

It is our contention that in selected cases, the operative procedure described by Vrabec and Hallberg (1) should be instituted as soon as the work-up is complete and the patient is in suitable operative condition. We limit the kind of leak to those which occur due to the pathology in the localized area of the cribriform plate, and not to intracranial causes such as tumor, pneumocephalus, or internal hydrocephalus. However, there may well be instances where this procedure can be used to close the leak, avoiding the often unnecessary complications of brain abscess and meningitis, and thus allowing intracranial procedures without the threat of retrograde infection.

Through a personal communication with Dr. Hallberg, we have been notified that he has performed several more of these procedures with excellent results. He stated that only one had a cerebrospinal fluid leak postoperatively, and the leak stopped when the cribriform area was lightly cauterized with 50% trichloroacetic acid and packed tightly with oxycel packing.

We have performed two additional operations for cerebrospinal fluid rhinorrhea through an intranasal approach. In the first of these, we found that the middle turbinate mucosa was tightly adherent to the middle turbinate bone. This appeared to be due to a marked cellularity on the medial surface of the middle turbinate. It is well known that the middle turbinate can be extensively pneumatized, and the mucosa seemed to invaginate into the many ethmoid cells present on the medial surface. The flap was torn, and free grafts of this mucosa were used rather than an advancement flap. In the next case, 1% Xylocaine® with Adrenalin was injected into the middle turbinate to create a better plane for dissection. The Zeiss operating microscope was used with the patient in a supine position, and exposure through a nasal speculum or an ear speculum was excellent. Gauze packing was placed between the lateral nasal wall and the lateral side of the middle turbinate. This prevented the middle turbinate from being freely mobile. This fixation of the middle turbinate greatly aided the dissection. The stapes knife and separator were used for this dissection. The patient in this case was a 3-year-old boy who suffered a skull fracture and had cerebrospinal fluid rhinorrhea for over 6 months. The operation has stopped this leak. The first of these two additional cases has leaked postoperatively, and is now being treated with cautery.

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Unusual Problems in Surgery

A. ROBERT BECK, M.D., AND JULIUS J. LEICHTLING, M.D., Co-Editors

Congenital Jejunal Diaphragm in a Premature Infant

Intestinal obstruction caused by the presence of an intraluminal diaphragm of the small bowel is uncommon in infancy. This lesion is a form of intestinal atresia in which a septum or diaphragm may completely or partially occlude the bowel lumen.

Case Report

B.G.B., #472224. A premature infant weighing 1,530 gm (3 lb 6 oz) was born on October 14, 1968 of a normal spontaneous delivery. The mother was a 27-year-old Negress, Para 2, Gravida 3, who had a positive VDRL, gestational diabetes, and iron deficiency anemia. The baby's condition at birth was fair; oxygen was given for a few minutes but no resuscitation was required. She was transferred to the Elmhurst Hospital Premature Unit at 5 hours of age. On arrival, the baby was in good condition, and physical examination, including neurological examination, was within normal limits. A complete blood count and urinalysis were normal. Later in the day some distention of the upper abdomen was noted and the baby began to regurgitate mucoid material. A nasogastric tube was inserted and approximately 15 cc of greenish material was aspirated from the stomach. An x-ray of the abdomen was taken at 8 hours of age, and air was seen only within the stomach and the duodenum (Fig. 1). Ten cubic centimeters of gastrografin was instilled into the stomach through a nasogastric tube. Films taken 40 minutes later demonstrated complete obstruction at the level of the upper jejunum (Fig. 2). At 15 hours of age the infant was taken to the operating room, and a laparotomy performed under general anesthesia. A congenital diaphragm completely obstructing the bowel lumen was found five centimeters distal to the ligament of Treitz. Proximal to the obstruction the bowel was markedly dilated, and distally the bowel was collapsed (Fig. 3). Saline was injected into the lumen of the bowel distal to the obstruction using a 25 gauge needle, and the patency of the bowel was established. A 2 cm longitudinal incision was made in the jejunal wall, half of the incision being above, and half below the lesion (Fig. 4). The web was then completely excised and the bowel closed transversely in two layers using 6-0 interrupted silk sutures. A gastrostomy was performed using a #14 Pezzer catheter. A #5 French polyethylene tube was inserted into the stomach through the gastrostomy opening and was then directed through the pylorus and duodenum and beyond the suture line to a point 10 cm distal to the ligament of Treitz (Fig. 5).

The baby did well postoperatively. The electrolytes were kept within balance by careful regulation of intravenous fluids and accurate replace-

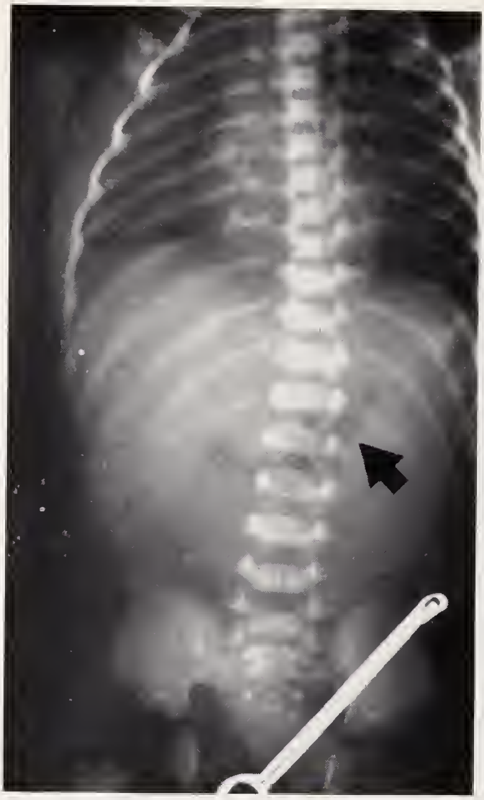

Fig. 1. X-ray at 8 hours of age. Infant in supine position. Gas is present in the proximal bowel only (arrow).

ment of the gastrostomy losses. On the third postoperative day the child had a stool, and by the fourth postoperative day the gastrostomy tube was draining only a few cubic centimeters of saliva. Gastrostomy feedings of glucose water were started on the seventh postoperative day. Oral feedings of formula were then given. A gastrointestinal x-ray study on the 13th postoperative day showed good function of the anastomosis, and the jejunostomy tube was removed. The gastrostomy tube was removed 1 week later. A second upper gastrointestinal series was performed just prior to discharge. This study showed free passage of barium into the distal bowel and much less dilatation of the jejunum proximal to the anastomosis. The baby was discharged at 7 weeks of age, at which time she weighed 5½ pounds. At 4 months of age the baby weighed 11 pounds and was doing well.

Discussion

Atresia of the small intestine is uncommon in the newborn, occurring once in every 10,000 to 20,000 births (1). The ileum is the most frequently involved area of the bowel (2). Multiple atresias occur in 10 to 15% of cases (1).

Three morphological types of intestinal atresia have been described

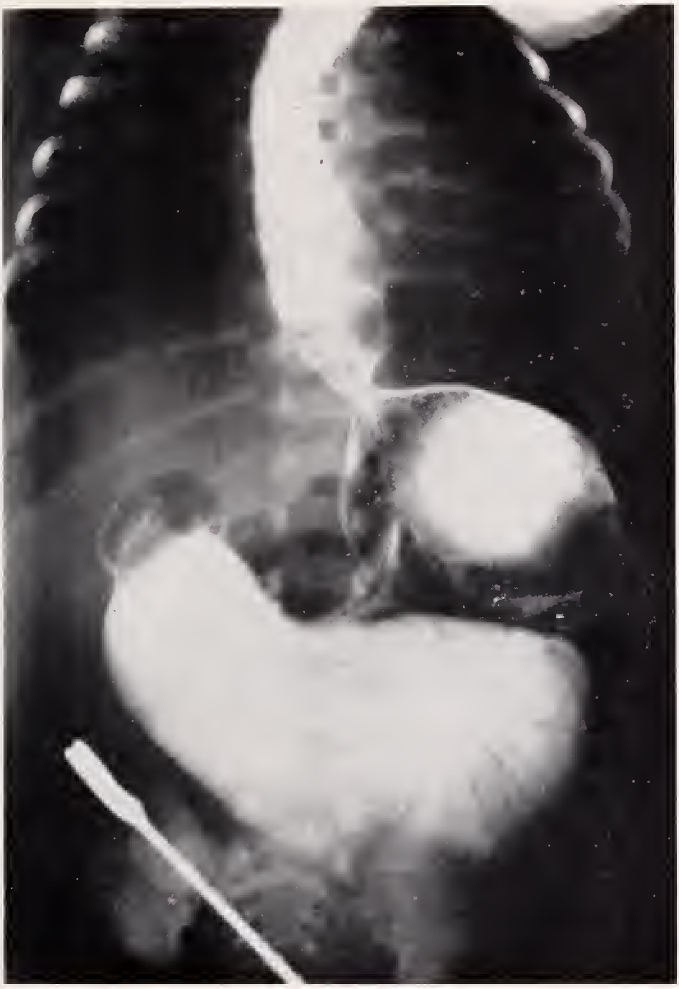

Fig. 2. X-ray taken 40 minutes after instillation of gastrografin. Note markedly dilated proximal jejunum, and no contrast material in the distal bowel.

(3). The most common variety is a complete separation of the dilated blind proximal bowel and the collapsed distal bowel, usually with a "V" shaped mesenteric defect between the two. In the second type, a cord-like connection is present between the proximal and distal bowel. The congenital web, or diaphragm, occurs less frequently than the other types.

Congenital atresia has long been regarded as an embryonal malformation caused by the lack of recanalization of the intestinal tube following the solid epithelial "plug stage" at the end of the second month of development (4). Further studies in human embryos (5) show that this process occurs most frequently in the duodenum of 5 to 8 week embryos, but is occasionally seen in the distal small bowel and colon. Since in clinical cases meconium is frequently found distal to a site of atresia, serious doubt has

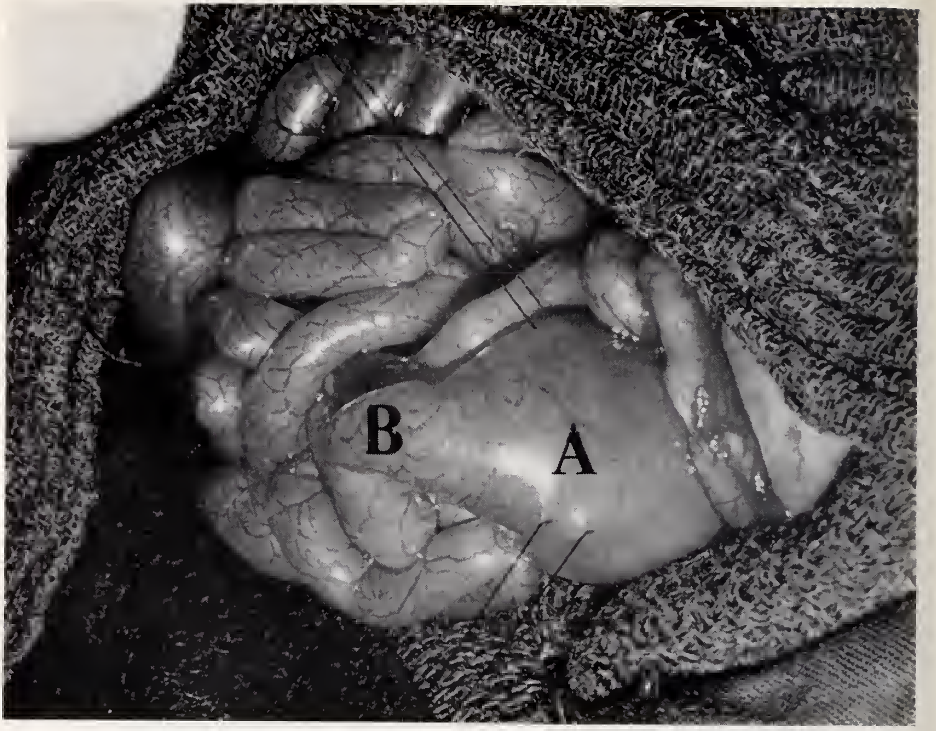

Fig. 3. Operative photograph taken after injection of saline into distal bowel. A = dilated proximal bowel; B = narrowed distal bowel. Web is located between the two guide sutures.

been cast upon the applicability of this explanation in all cases of intestinal atresia. There have been isolated case reports in which specific lesions have been implicated as the cause of intestinal atresia. Among these are snaring of the intestine by the umbilical ring (6), intussusception (7), and volvulus (8). In 1955, Louw and Barnard (8) showed that intestinal atresia can be produced by a vascular accident in a previously normally developed fetal intestine. Fetal puppies were operated upon *in utero*, and the vessels to a short segment of the small bowel were ligated. Examination of the pups after birth showed that intestinal atresia, both of the blind end and diaphragm type, had been produced in several animals. Intrauterine vascular accidents are now thought to be the probable cause in most cases of intestinal atresia.

The cardinal signs of intestinal atresia are bilious vomiting, abdominal distention, and failure to pass meconium. The degree of distention varies with the location of the lesion; the more distal the site of atresia, the greater the degree of distention. Prompt diagnosis and therapy are essential for a successful outcome. Abdominal x-rays of the infant in the supine and upright position should be taken. If there is definite evidence of a high ileal or jejunal obstruction, no

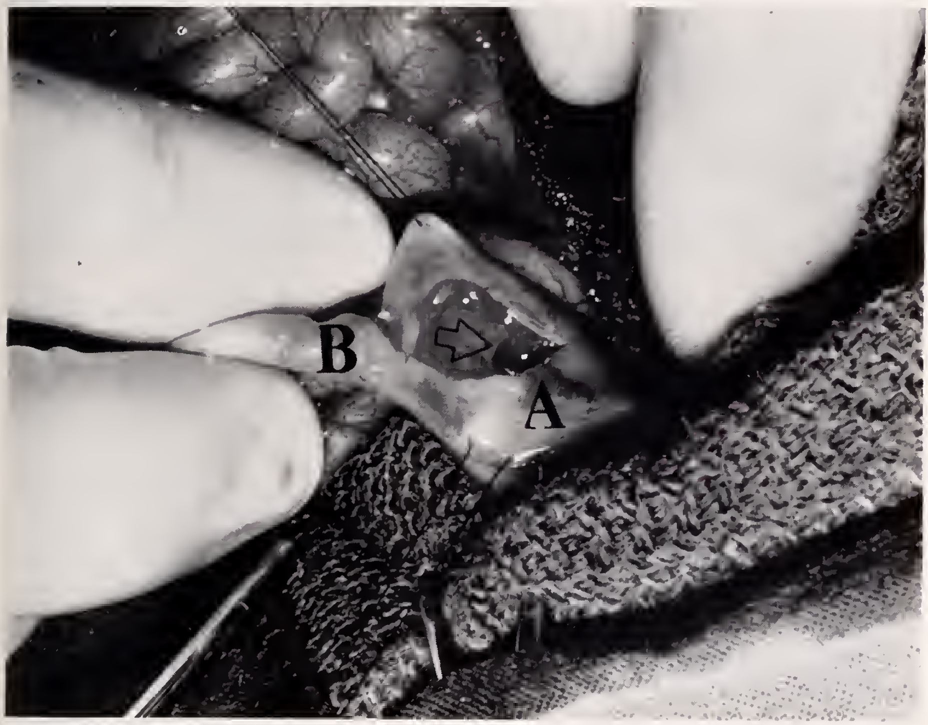

Fig. 4. Operative photograph. Bowel has been opened. A = proximal bowel; B = distal bowel. Web is indicated by arrow.

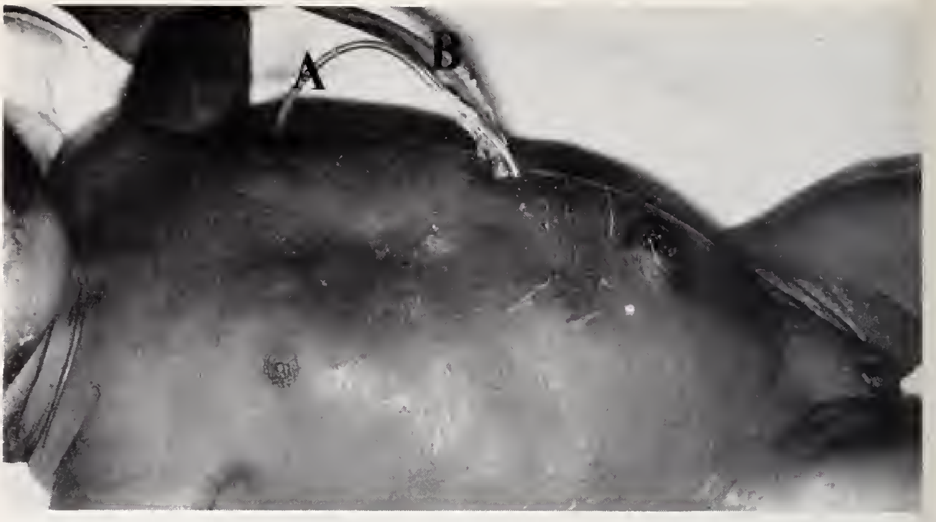

Fig. 5. Postoperative photo showing the placement of gastrostomy tube (B) and jejunostomy tube (A) (see text for details).

further films are needed. However, in the presence of a low intestinal obstruction a barium enema must be done in order to differentiate low ileal atresia from meconium plug (a nonsurgical condition) and Hirschsprung's disease. In duodenal obstruction further studies are necessary to distinguish annular pancreas and duodenal atresia from malrotation, as the latter requires immediate surgery.

The best results in the treatment of intestinal atresias and webs have been obtained with a liberal resection of the dilated bulbous proximal bowel and a primary end-to-end anastomosis (9). Nixon (10) has shown that the dilated end of the proximal bowel lacks normal peristaltic activity, and therefore resection must be performed in relatively normal bowel if the anastomosis is to function properly. Usually 10 to 15 cm of the proximal bowel must be removed. In the case presented, the obstructing lesion was so close to the ligament of Treitz that a liberal proximal resection was impossible, and it seemed wise to simply remove the web and perform a jejunoplasty. The use of a small polyethylene catheter, which is introduced through the gastrostomy opening and is placed distal to an anastomosis, has been described in high jejunal atresias (11). It can be used for replacement of gastric drainage, as well as for feeding, when delayed function of the anastomosis occurs.

Summary

A successfully treated case of intestinal obstruction due to a jejunal web in a premature infant is described. A general consideration of intestinal atresia, as well as its possible etiology, clinical presentation, and surgical treatment is discussed.

A. Robert Beck

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RADIOLOGICAL NOTES

CLAUDE BLOCH, M.D., AND HARVEY M. PECK, M.D., Co-Editors

SUBMITTED AND DISCUSSED BY JOHN C. LEONIDAS, M.D.

CASE NO. 337

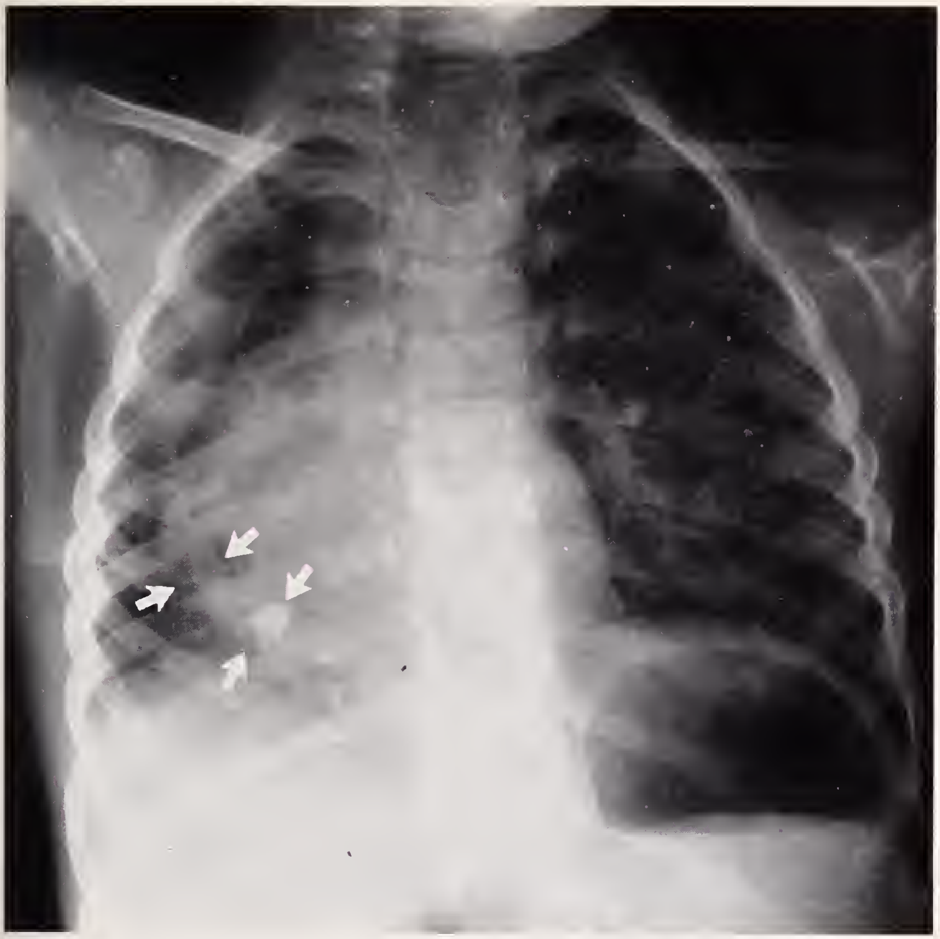
This 3-year-old female was seen in the Pediatric Clinic for investigation of suspected dextrocardia and a heart murmur. Past history was uneventful. The patient was entirely asmyptomatic. Physical examination revealed a well nourished and well developed female child in no distress. Heart sounds were best heard to the right of the sternum. S_2 was split. A grade 2/6 holosystolic murmur was heard along the left sternal border, left axilla, and back. The electrocardiogram was consistent with dextrocardia.

A chest roentgenogram was obtained as part of the cardiologic evaluation (Fig. 1). The right lung was definitely smaller than the left, and there was dextroposition of the heart and elevation of the right hemidiaphragm. The abdominal viscera were normal in position. The aortic arch was on the left. Adjacent to the right cardiac border, and partially obscured by the heart, a vertical vascular structure could be identified descending parallel to the heart border, and increasing in size as it approached the right diaphragm (between arrows); it formed a gentle curve with its convexity to the right (scimitar).

The findings were characteristic of the "Scimitar Syndrome" (1), and the patient was admitted for confirmation of the diagnosis and investigation of possible coexisting congenital intracardiac anomalies. Cardiac catheterization disclosed oxygen saturation step-up in the low right atrium, indicative of left-to-right shunt. Pressures were normal. Selective angiocardiography was performed with injections of contrast in the right ventricle and main pulmonary artery. The findings are seen in Figure 2 (right ventricular injection) and Figures 3A and 3B (Pulmonary arterial injection); a detailed anatomic description is given in the legends of the angiocardiograms.

Discussion

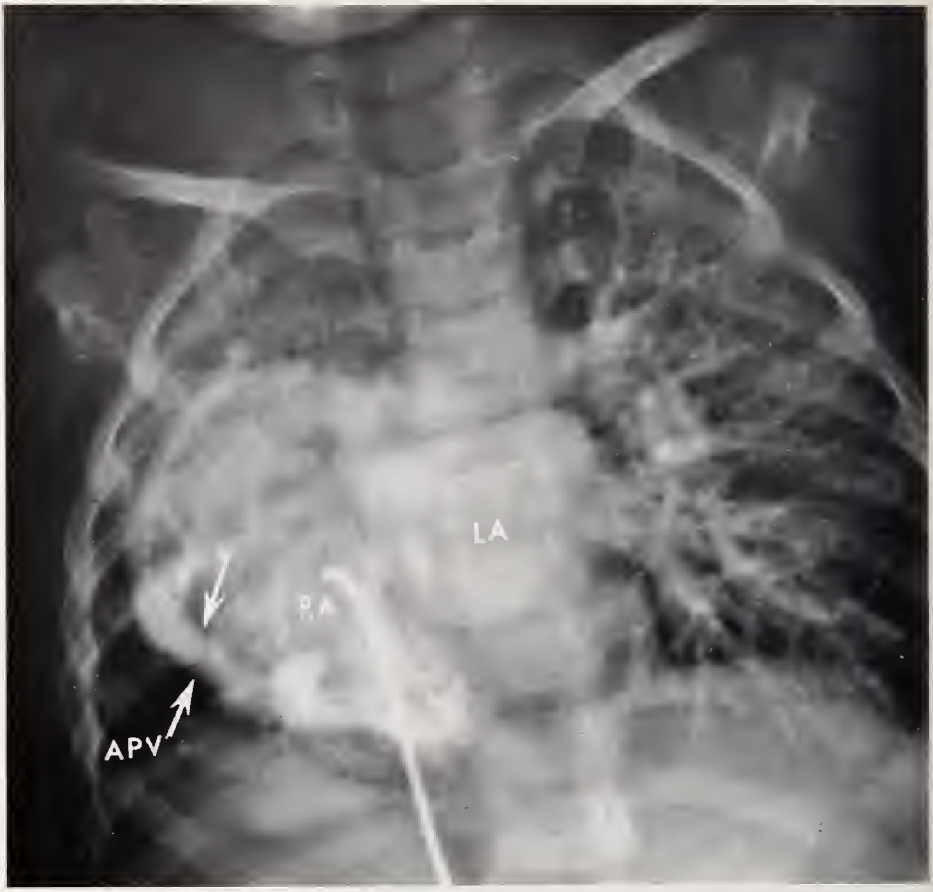
The roentgenographic features of the syndrome of anomalous pulmonary venous return of the right lung associated with ipsilateral pulmonary hypoplasia are very characteristic. They are well illustrated in the case described above. The syndrome complex consists of: (1) anomalous pulmonary venous drainage either of the entire right lung, or of the right middle and lower lobes into the inferior vena cava. The draining vessel descends toward the diaphragm in the right paracardiac area and joins the inferior vena cava below the diaphragm; (2) hypoplasia of the right lung with bronchial anomalies; (3) dextroposition or dextrorotation of the heart; and (4) anomalous systemic arterial



Case 337, Fig. 1. Posterioanterior roentgenogram of the chest. The heart is displaced to the right (pulmonary hypoplasia). A smooth curvilinear density at the right cardiac border (between arrows) is partially obscured by the shadow of the heart; it represents a vascular structure descending toward the right dome of the diaphragm. This vessel is the right pulmonary vein which crosses the diaphragm to drain abnormally into the inferior vena cava (see text).

blood supply to the right lower lobe from the aorta by a vessel with subdiaphragmatic origin (1).

The syndrome is not very common. Halasz and his associates (2) pointed out the similarity of the anomalous vessel to a scimitar (saber with a curved blade with sharp edge on convex side, used mainly by Arabs and other Mohammedans). The term "Scimitar Syndrome" was coined by Neil et al in 1960 (3). Of 58 cases reviewed by Jue and associates, 22 were in the pediatric age group. There is a female preponderance (19 of 22 patients). Eight pediatric patients

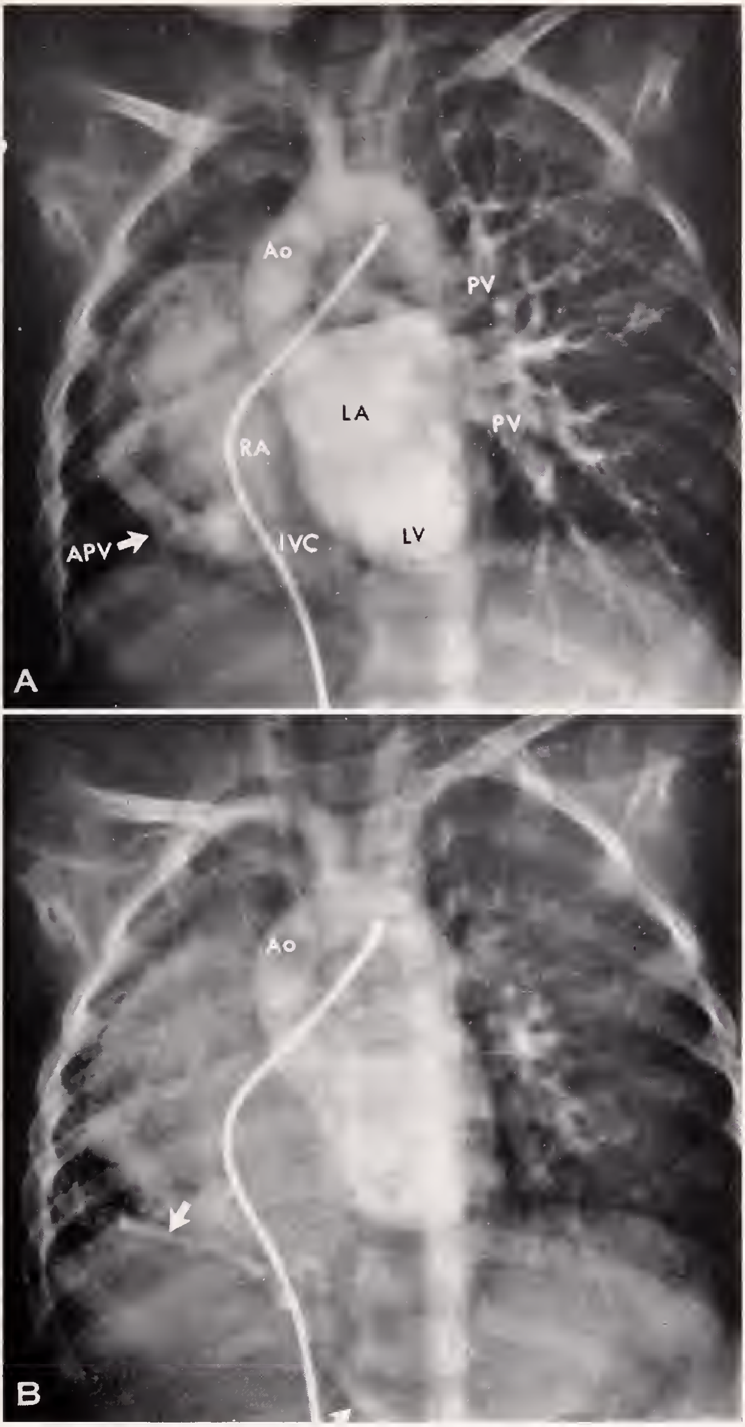


Case 337, Fig. 2. Selective angiocardiography, right ventricular injection. Late phase, with opacification of the left atrium (LA). The left pulmonary veins normally enter the left atrium; the right pulmonary venous return opacifies the previously described anomalous vein (APV) (arrows), through which the inferior vena cava and right atrium fill with contrast. RA = right atrium.

Case 337, Fig. 3A. Selective angiocardiography, injection into the pulmonary artery. During this phase, there is opacification of pulmonary veins and left cardiac chambers.

The left pulmonary veins (PV) converge and enter the left atrium (LA). There is no evidence of venous connection between the right lung and left atrium. The gently curved single anomalous right pulmonary vein (APV) drains the entire right lung into the inferior vena cava (IVC). RA = right atrium; LV = left ventricle; Ao = aorta.

Case 337, Fig. 3B. On this delayed angiocardiographic phase, a large subdiaphragmatic artery (arrows) originates from the descending aorta to supply the right lower lung. Ao = aorta.


Ao
PV
LA
PV
RA
APV
IVC
LV
A
Ao
B

Case 337, Figs. 3A and 3B

had associated congenital heart disease (ventricular septal defect, patent ductus arteriosus, coarctation of the aorta, tetralogy of Fallot) (1).

Many patients are asymptomatic and the syndrome is discovered on a roentgenogram obtained either routinely, or for investigation of clinical suspicion of dextrocardia. At other times, particularly with severe degrees of right pulmonary hypoplasia, these patients have recurrent pulmonary infection and sometimes evidence of pulmonary vascular hypertension (3).

The plain film findings are very characteristic. In the majority of cases, the gently curved anomalous vein descends vertically toward the diaphragm in the right paracardiac region (Fig. 1—arrows). With marked pulmonary hypoplasia, it may be seen behind the cardiac silhouette. The right lung is small and the heart is seen shifted to the right. Bronchography usually shows various anomalies of bronchial branching and pulmonary segmentation. The features of the syndrome are superbly demonstrated by angiocardiography. The right pulmonary artery is hypoplastic and the heart is rotated, so that the two ventricles are side by side in the AP view with the interventricular septum in a sagittal plane. Later, during pulmonary venous opacification, the left pulmonary veins enter the left atrium (Fig. 2). On the right side, the anomalous "Scimitar" vein fills with contrast from right pulmonary venous tributaries, and it may drain the entire right lung. Through this vein, the inferior vena cava and right atrium opacify. At times, the right upper pulmonary veins may drain normally into the left atrium or abnormally into the right atrium via the azygos vein, or directly into the superior vena cava (Fig. 3A).

In a significant number of cases, as well as in our case, subdiaphagmatic systemic arterial supply to the right lower lobe is present (Fig. 3B), in a fashion similar to the syndrome of congenital bronchopulmonary-foregut malformation (4) or sequestration. Embryologically, the anomaly can be explained by persistence of the early communication between the primitive pulmonary venous plexus and the splanchnic plexus in association with a fundamental developmental abnormality of the right lung.—C.B. and J.C.L. .

Case Report: "SCIMITAR SYNDROME."

Acknowledgment

This case is presented through the courtesy of Dr. Irwin Gribetz.

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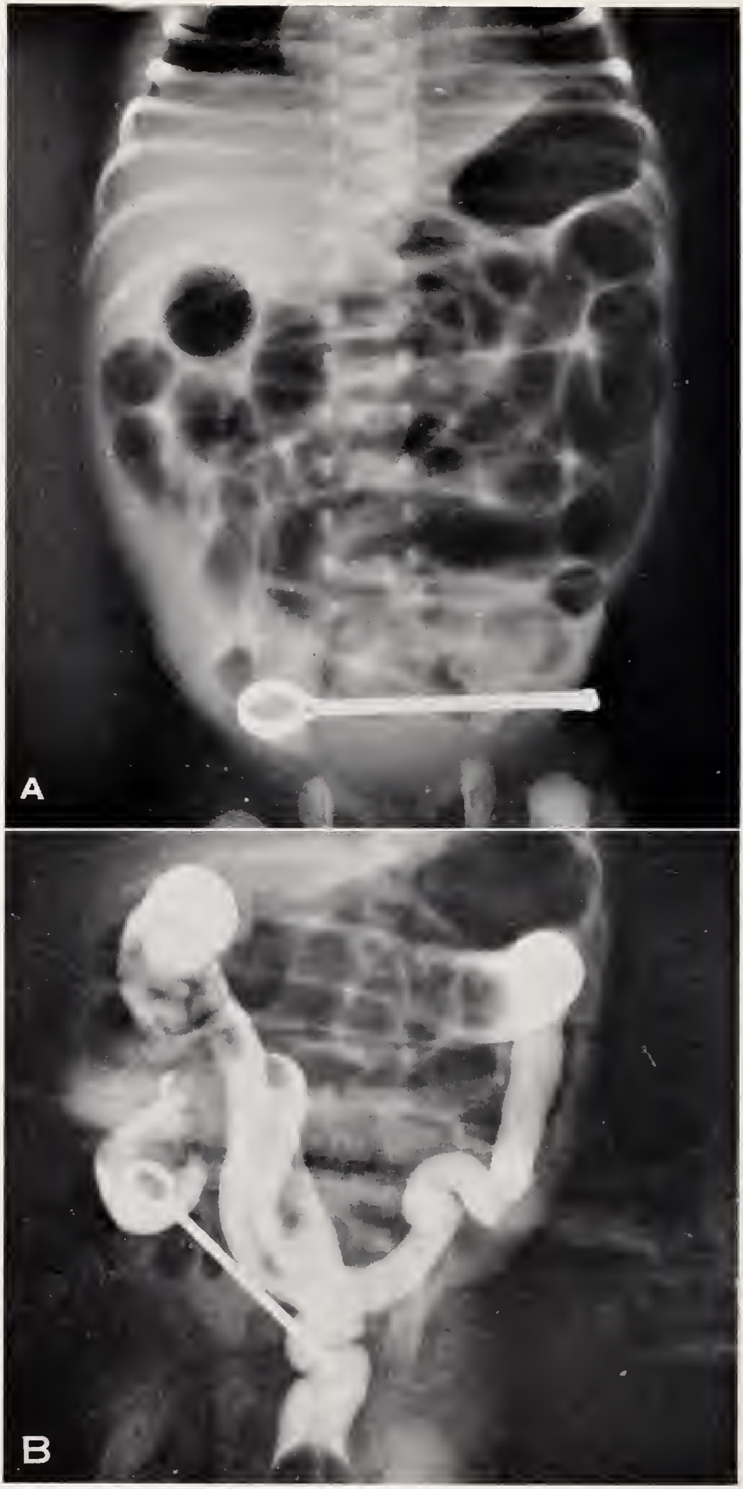
CASE NO. 338

This female infant was born after a 36 week gestation and weighed 2,270 gm at birth. The baby did not pass meconium and developed abdominal distension on the next day. Plain film of the abdomen showed multiple distended intestinal loops occupying the entire abdomen (Fig. 1A). The pattern suggested a low small bowel or colonic obstruction. A gastrographin enema was performed on the same day (Fig. 1B) which revealed a relatively narrow left side of the colon up to the splenic flexure. A long radiolucent column extending from the recto-sigmoid to the descending colon was thought to represent a mass of meconium. The caliber of the colon changed proximal to the splenic flexure, with the transverse and ascending colon being relatively dilated. A large meconium cast was noted to fill almost the entire transverse colon and extend proximally to the cecum (Fig. 1B). The findings were characteristic of the "meconium plug" syndrome (1). Following this study, the patient failed to improve. A laparotomy was performed on the third day of life which showed the left colon to be narrowed and the proximal colon dilated. Biopsy specimens were obtained from the sigmoid colon and splenic flexure, and frozen sections were reported to show no ganglion cells in the sigmoid, with normal ganglion cells in the proximal specimen (aganglionosis). A transverse colostomy was performed. Paraffin sections of the biopsy specimens of the colon revealed normal ganglion cells, and the initial diagnosis of aganglionosis (Hirschsprung's disease) was not tenable. Fifteen months later the colostomy was closed. Follow-up examinations have shown no intestinal problems.

Discussion

This case emphasizes the difficulty commonly encountered in the differential diagnosis between colonic aganglionosis and the "meconium plug" syndrome. Both entities become manifest at birth with signs of intestinal obstruction. Plain films of the abdomen are not helpful; usually there is generalized distension with muliple air-fluid levels, and often it is extremely difficult to differentiate small bowel from colonic loops, and to therefore determine the level of obstruction. Contrast studies of the colon should be the next step in the diagnostic work-up of these patients. A total microcolon, if present, will immediately rule out colonic obstruction, and the diagnosis of meconium ileus or ileal atresia should then be made. A normal appearing colon will favor the diagnosis of aganglionosis; delayed films to assess the rate of evacuation of barium over the next 24–48 hours should be obtained. If the patient does not improve clinically during this interval and there is very slow evacuation of contrast, then Hirschsprung's disease is the most likely diagnosis. Obviously a transition zone between the relatively narrow rectum and dilated sigmoid, whenever present at this early age, provides direct evidence of aganglionosis. Often the contrast enema will show a narrow left colon, with change of caliber in the region of the splenic flexure. A "meconium plug" is seen to fill the distal segment of the dilated



Case 338, Figs. 1A and 1B

colon. Under these circumstances, the provisional diagnosis of "meconium plug" syndrome should be made. The possibility of aganglionic megacolon cannot be excluded, however, and the clinical course along with delayed films will be extremely valuable for a final diagnosis.

The typical patient with "meconium plug" syndrome evacuates the plug following barium enema, and he does well thereafter. In some cases, however, a clear-cut differential between Hirschsprung's disease and meconium plug is not possible; the patient remains with signs of colonic obstruction, and a temporary colostomy and/or rectal biopsy should be performed.

"Meconium plug" syndrome is a symptom complex of temporary colonic obstruction in the newborn, the etiology of which is obscure (1, 2). The most distal segment of the meconium mass is firm, granular, and pale yellow, and there is alteration of the chemical composition of this terminal portion of intestinal content (3); reduced tryptic activity has been reported (1, 2). Evidently the newborn encounters difficulty in expelling this plug. These patients do not have cystic fibrosis. In that disease, the meconium inspissates in the terminal ileum (meconium ileus). Relief of obstruction in "meconium plug" syndrome is usually achieved by introduction of a thermometer, rectal examination, and contrast enemas. The final diagnosis, however, is one of exclusion of aganglionosis.—C.B. and J.C.L.

Case Report: "MECONIUM PLUG" SYNDROME.

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CASE NO. 339

This 3,740 gm male infant was the product of a normal full term gestation. Soon after birth, progressive abdominal distension was noted and the patient vomited bile-stained material. A small amount of meconium was passed. Physical examination revealed a tense, markedly distended abdomen. Bowel sounds were absent. The rectum was empty on digital examination. A supine plain film of the abdomen showed uneven distension of intestinal loops, traversing

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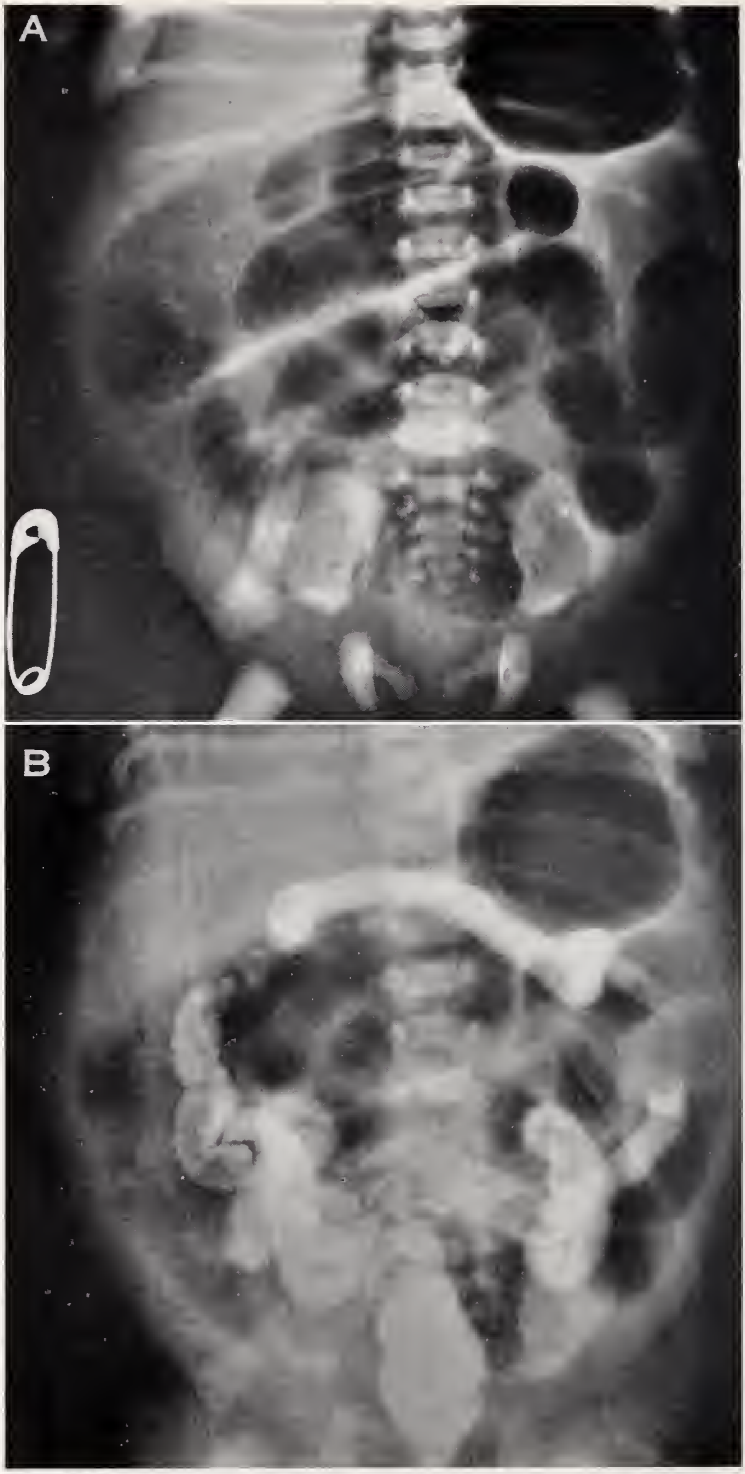
Case 338, Fig. 1A. Plain anteroposterior film of the abdomen in the supine position. Generalized gaseous distension of bowel; differentiation between small bowel and colonic loops virtually impossible.

Case 338, Fig. 1B. Barium enema shows a relatively narrow left colon up to the splenic flexure. Proximal to this point bowel becomes wide. Plugs of meconium fill both the sigmoid and transverse colon (intraluminal lucencies). The findings are typical of the "meconium plug" syndrome as defined by Clatworthy.



Case 339, Figs. 1A and 1B

the abdomen in parallel or step-ladder fashion (Fig. 1A). To confirm the impression of small intestinal obstruction, a barium enema was performed. A normally rotated microcolon was filled (Fig. 1B), establishing the diagnosis of antenatal small bowel obstruction (1). Laparotomy was performed, and jejunal atresia was discovered. An end-to-side jejunojejunostomy, cutaneous jejunostomy, and gastrostomy were performed, and the patient made an uneventful recovery. The resected surgical specimen strongly suggested prenatal intussusception.

Discussion

The most common cause of intestinal obstruction in the newborn is a congenital intrinsic lesion of the alimentary tract. Jejunal or ileal atresia and stenosis are considered to be the result of intrauterine interference with the blood supply of a segment of the bowel, usually secondary to prenatal volvulus (2); this type of congenital intestinal obstruction often complicates meconium ileus (3), and it is suggested that exploration of the terminal ileum for the typical pellets of inspissated meconium should be carried out on all patients undergoing surgery for intestinal atresia.

In atresia, there is complete loss of continuity of the lumen of the bowel. The proximal dilated segment of the atretic small bowel ends blindly. There may be complete separation between the proximal and distal segments, often accompanied by a defect in the mesentery, or there may be a connecting solid fibrous cord extending between the two segments (4). Complete intraluminal diaphragms are occasionally found. Prenatal volvulus, leading to atresia because of ischemia of the twisted loop, may cause atresia associated with intestinal "pseudocyst" (5). Prenatal intussusception was the probable mechanism of ischemia leading to atresia in the case presented.

The "bubbly" pattern of retained meconium and intestinal gas in the obstructed small intestine noted in this case (Fig. 1A) was originally described as a specific Roentgen sign of meconium ileus (6). Later, the nonspecificity of the sign became evident, when a similar pattern was noted in aganglionosis, imperforate anus, meconium plug, and ileal atresia. Uneven distension of small bowel loops, "bubbly" pattern, and microcolon in this case confidently narrow down the list of differential diagnosis to low jejunal or ileal atresia and meconium ileus.—C.B. and J.C.L.

Case Report: Low jejunal atresia.

Case 339, Fig. 1A. Plain anteroposterior film of the abdomen in the supine position. Multiple distended loops of bowel. Level of obstruction cannot be determined on the basis of plain roentgenogram. Note "bubbly" appearance of distended right upper quadrant loop, a nonspecific sign of retention of meconium.

Case 339, Fig. 1B. Barium enema demonstrates microcolon. It now becomes evident that distended loops are small bowel. Differential diagnosis limited between jejunoileal atresia and meconium ileus. On erect films (not shown) air fluid levels were present, a finding suggestive of atresia.

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CASE NO. 340

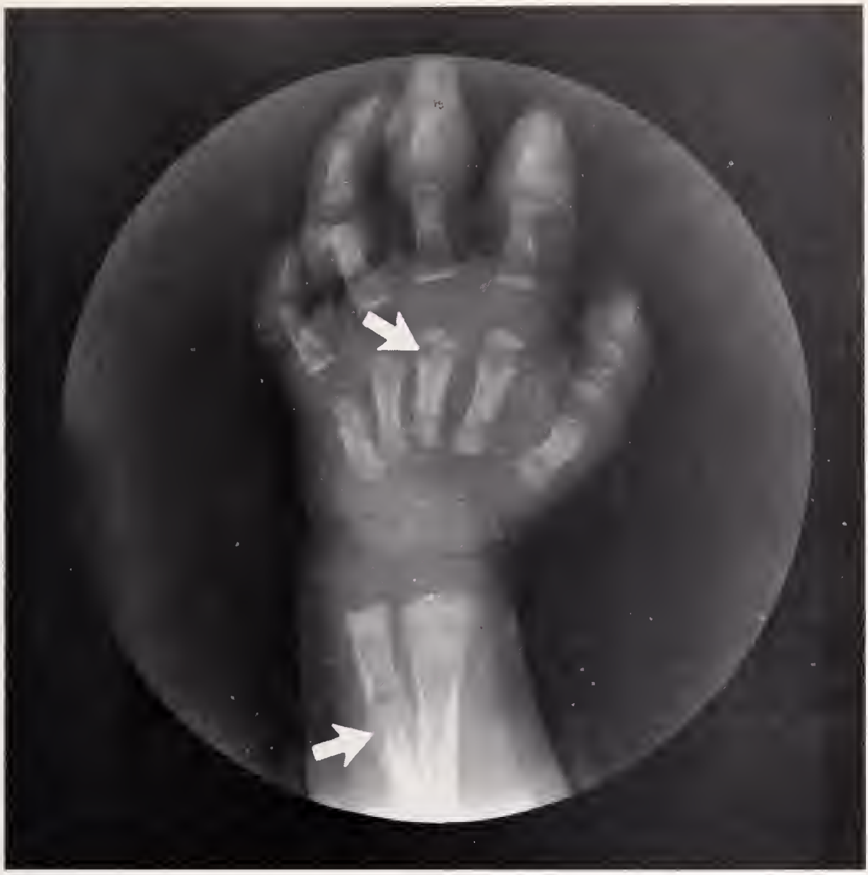
This 6-week-old white male infant was admitted with a 3 week history of anorexia, fever, and edema. He was the product of a full term pregnancy and normal spontaneous delivery. The early neonatal history was unremarkable. Three weeks prior to admission, the patient developed fever and a transient macular rash. Anorexia, pallor, and irritability were noted soon thereafter. Edema of the hands and feet appeared later in the course of illness. Physical examination on admission revealed pallor, periorbital edema, and fusiform swelling of all his fingers. There was also edema of the dorsum of the hands and feet, hypotonia, and hyperreflexia. The hemoglobin was 6.9 gm, white blood count 12,000/mm^3, with a normal differential count, and erythrocyte sedimentation rate 48 mm/hour. Reticulocyte count was 4.1% and platelets numbered 854,000/mm^3. A lumbar puncture yielded normal cerebrospinal fluid. Bone marrow revealed moderate erythroid hyperplasia. Sickle cell preparation and tuberculin tests were negative.

Roentgenographic examination of the long bones of the extremities showed bilaterally symmetrical changes of bone destruction and production, involving the metacarpals and phalanges (Fig. 1). There was patchy rarefaction of the entire shaft of the small tubular bones of the hands, with irregular reactive sclerosis and periosteal new bone formation (arrows). The distal ulna and radius showed submetaphyseal lucent zones of growth disturbance, periosteal cloaking, and irregular bone destruction of the shaft. There was fusiform soft tissue swelling surrounding the osseous lesions of the hands. Similar bone changes were noted in the metatarsals and the distal tibiae and fibulae. The rest of the skeleton showed only minimal changes, namely periosteal new bone of the distal humeri and femora.

Serological tests for syphilis were strongly positive for the patient and his mother. Immunoelectrophoresis showed marked elevation of IgA and IgM immunoglobulins.

Discussion

Congenital osseous syphilis has become a rare disease over the last few decades, and most cases now seen by the radiologist in training are limited to



Case 340, Fig. 1. Left hand. Note severe dactylitis of metacarpals and phalanges, as well as involvement of distal ulna and radius (arrows). There is submetaphysial bone destruction and periosteal new bone production. Changes were bilateral and symmetrical.

"x-ray teaching files." In recent years the incidence of syphilis has been increasing, however, and one may expect to encounter the congenital form of this disease more often. Syphilis should always be considered in the differential diagnosis of extensive early infantile osteomyelitis, and a very high index of suspicion should be maintained in the presence of bilaterally symmetrical lesions.

The metacarpals, metatarsals, and phalanges are infrequently involved in congenital syphilis. Dactylitis is very rare in the first few months of life, except when the long tubular bones of the extremities are also involved (1). The marked syphilitic changes of the distal extremities, and particularly the severe dactylitis associated with only minimal changes of the more proximal long bones, is an unusual feature in our case.

The roentgenographic appearance of syphilitic dactylitis is nonspecific, and one has to rely on associated clinical and laboratory findings, or the x-ray changes of other parts of the skeleton, for correct diagnosis. The differential diagnosis includes the "hand and foot syndrome" of sickle-cell disease (2), tuberculous dactylitis (spina ventosa), and pyogenic osteomyelitis, usually associated with septicemia at this age. Dactylitis of a similar roentgenographic appearance has also been seen in acute leukemia in children (3). The characteristic distribution of the osseous manifestations of congenital syphilis in a bilaterally symmetrical fashion, usually coupled with the typical syphilitic changes of the long tubular bones of the extremities, may lead the radiologist to the correct diagnosis (1, 4).—C.B. and J.C.L.

Case Report: CONGENITAL OSSEOUS SYPHILIS WITH SEVERE DACTYLITIS.

Acknowledgment

This case was referred to The Mount Sinai Hospital by Dr. Eugene Wasserman of Mamaroneck, New York.

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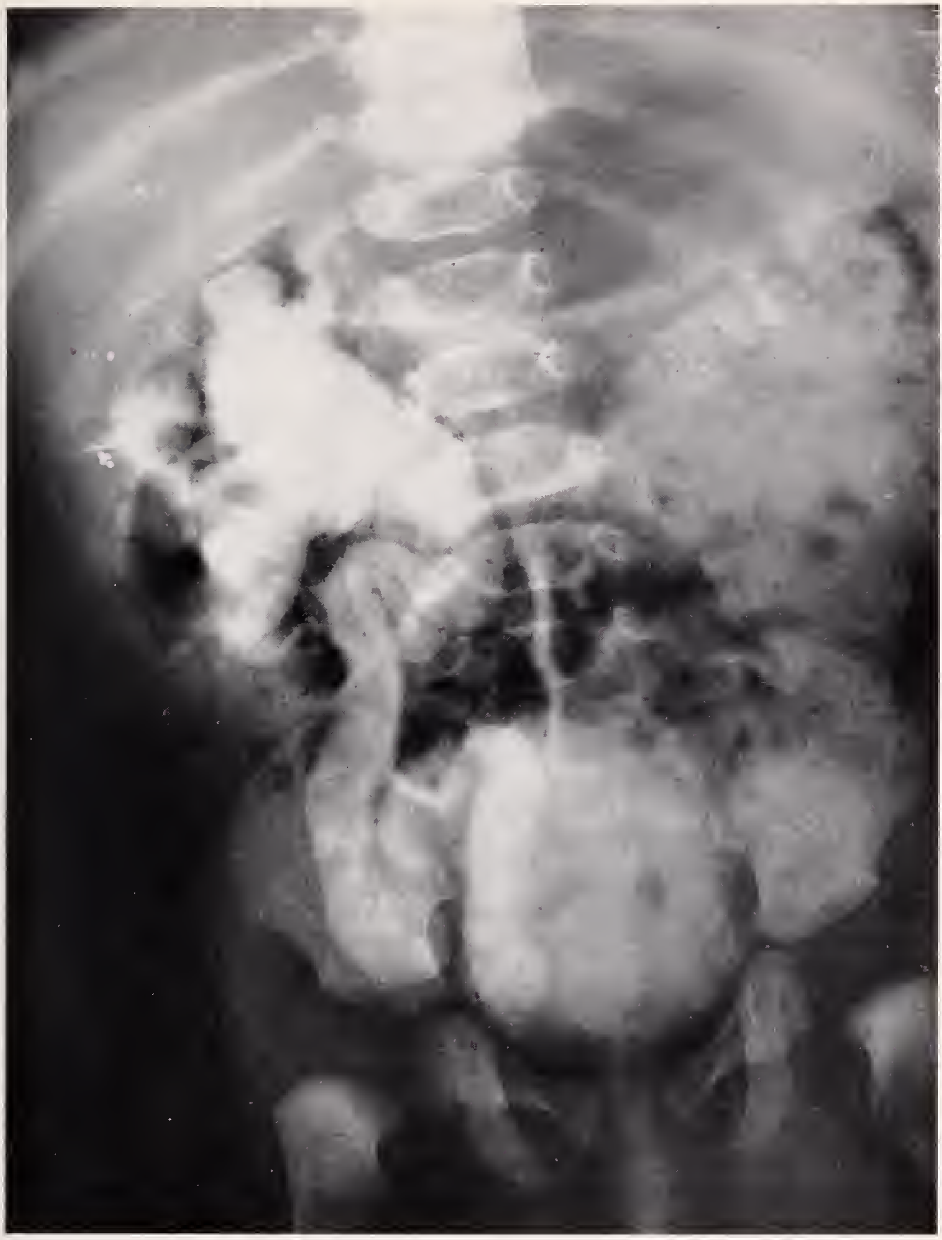
CASE NO. 341

A 5-month-old male infant was admitted to the hospital with a 3 day history of fever, vomiting, and diarrhea. Past history was unremarkable. Physical examination revealed poor growth and development. No abdominal masses were palpated. Bladder outlet obstruction was suspected on the basis of poor urinary stream.

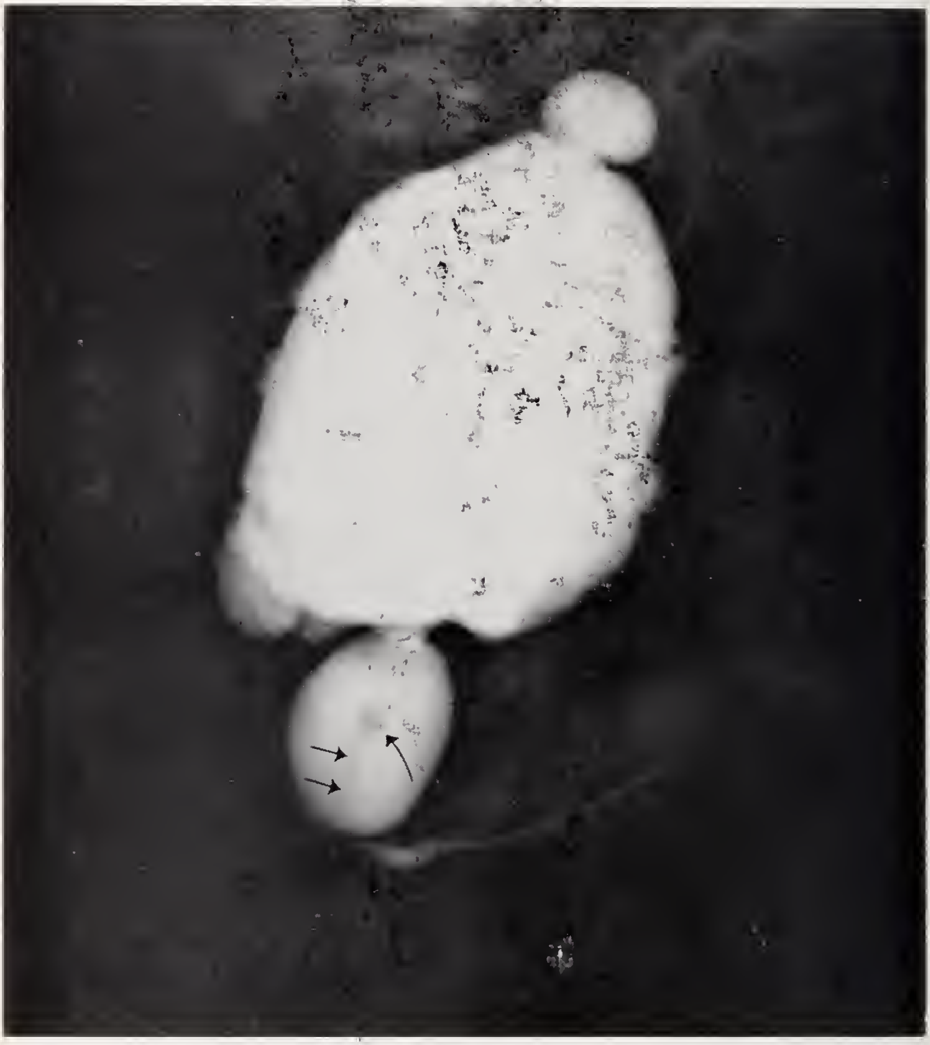
Laboratory values on admission were hematocrit 34%, and creatinine 1.3 mg%. Urine was cloudy, loaded with pus cells and bacteria. Urine and blood cultures revealed Aerobacter aerogenes. The findings established the diagnosis of urinary tract infection and bacteremia associated with obstructive uropathy.

Intravenous pyelogram (Fig. 1) showed marked hydronephrosis and hydroureter on the right, with normal excretion of contrast. There was poor opacification of the left collecting system due to poor renal function. Catheterization of the bladder revealed significant urinary retention. A retrograde cystogram showed a large decompensated bladder, markedly trabeculated, and with multiple cellules. A diverticulum was noted at the dome of the bladder. The voiding cystourethrogram showed a relatively narrow bladder-neck area and dilatation of the posterior urethra. The characteristic longitudinal linear lucency in the region of the verumontanum was present, distally forming thin



Case 341, Fig. 1. Intravenous pyelogram. One and one-half hours following injection of contrast medium, film shows marked bilateral hydronephrosis. Ureters are dilated and tortuous. Findings indicate bladder outlet obstruction and/or massive bilateral vesicoureteral reflux.

folds merging with the smooth, curved, ballooned-out margins of the posterior urethra. This is the typical appearance of posterior urethral valves (Fig. 2—arrows). The presence of valves was confirmed by endoscopic examination.



Case 341, Fig. 2. Voiding cystourethrogram following retrograde contrast filling of the bladder, which appears trabeculated. There is one diverticulum at the dome, and a second at the base of the bladder, on the right side of the trigone. The posterior urethra is markedly dilated. The arrows point to the lucent folds which arise in the region of the verumontanum (single arrow), and extend inferiorly (double arrows) to obstruct the flow of urine; this is the typical appearance of the most common type of posterior urethral valves. Distal to the valves, stream of urine in anterior urethra markedly attenuated.

Discussion

Posterior urethral valves constitute the most common cause of bladder outlet obstruction in the male infant. Young described three types of posterior urethral valves. Type I is by far the most common form. Mucosal folds extend from the verumontanum or from the midline dorsal ridge on the urethra downward and laterally, and usually fuse anteriorly (1, 2). They resemble the cusps of the aortic valve, permitting retrograde passage of a catheter, yet they are obstructive to the antegrade flow of urine (3). Type II valves are uncommon, and it is questionable whether they represent true valves (1). In type III, there is a diaphragm with a central opening of variable diameter obstructing the posterior urethra.

As a result of the chronic obstruction, the posterior urethra dilates considerably. The finding is best demonstrated during voiding cystourethrography (Fig. 2), at which time the folds are often visualized as linear lucencies originating from the verumontanum. Bladder hypertrophy leads to an accentuation of the indentation caused by the "bladder-neck." In addition, the bladder becomes irregular, trabeculated, and it may form cellules and diverticula. Vesicoureteral reflux, unilateral or bilateral, may be present. There is bilateral hydronephrosis, usually more severe in the presence of reflux, and occasionally renal dysplasia. Impairment of renal function accompanies long standing severe forms of obstruction, and urinary tract infection is commonly present and usually leads to the original investigation of the urinary tract. Early diagnosis and management are crucial in terms of preservation of renal function and ultimate survival. Unfortunately, symptoms and signs rarely point to the urinary tract in the newborn or infant, and such nonspecific symptoms as vomiting, diarrhea, anorexia, failure to thrive, or even ascites may be the presenting features. Fever of "unknown origin" may be the only manifestation of urinary tract obstruction. Complete investigation of the urinary tract should be carried out as soon as possible in the presence of these nonspecific findings in the infant and young child.

Management of posterior urethral valves is not simple, and thorough description of available therapeutic technics and measures is beyond the scope of this brief presentation. The goal is adequate resection of the valves; however, renal decompensation, infection, reflux, dilatation, and tortuosity of the ureters, and bladder hypertrophy are some of the problems that have to be dealt with during treatment.—C.B. and J.C.L.

Case Report: POSTERIOR URETHRAL VALVES (TYPE I).

Acknowledgment

The author expresses his appreciation to Dr. Elliot Leiter for contribution of material of this case.

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OPHTHALMOLOGICAL NOTES

THEODORE W. LIEBERMAN, M.D., EDITOR

Neuro-Ophthalmologic Signs and Symptoms of Occlusive Disease of the Carotid and Vertebral Arteries

By STUART E. WUNSH, M.D., AND THEODORE W. LIEBERMAN, M.D.

Visual symptoms and signs are frequently associated with occlusive disease of the carotid and vertebral-basilar arterial systems. Most symptoms are caused by local tissue ischemia either of the eye or of the visual pathways. If transient, such events may be inconsequential. However, their occurrence may presage permanent ocular or central nervous system damage. This paper reviews the visual system changes associated with occlusive disease of the cervical arterial supply to the central nervous system.

1. Occlusive Disease of the Carotid Artery. The ocular symptoms and signs of carotid vascular disease can be divided into two categories according to whether intermittent or chronic vascular insufficiency predominates:

A. *Transient Vascular Insufficiency.* Transient ischemic attacks (TIA) are temporary episodes of neurologic deficit. When confined to the distribution of one of the carotid systems, they are expressed clinically as episodes of motor or sensory deficits contralateral to the involved carotid, aphasia, and/or ipsilateral visual changes. The symptoms and signs of such an attack usually last minutes, but may endure several hours. When involving the eye, the attack has been termed amaurosis fugax (fleeting blindness). The patient describes a curtain or shade obscuring part of the vision of one eye or transient total monocular loss of vision. Less frequently, a transient homonymous hemianopsia may occur from ischemia of the optic tracts or radiations which in part are supplied by the carotid system. In an ophthalmologist's series (1) of 235 patients with symptomatic involvement of the carotid system, 206 patients, or 88%, had ocular signs or symptoms. In a neurologist's series (2) of 181 patients, 41, or 21.9%, had visual disturbances.

The pathogenesis of all transient ischemic attacks is not known. In the older age group it is commonly associated with atheromatous disease of the carotid or vertebral arteries in the neck. With a high degree of stenosis of these arteries, hypotensive or vasoconstrictive episodes may cause transient local ischemia. Currently popular is the opinion that attacks are caused by embolization of material from an atheromatous plaque to local vasculature. Despite the attractiveness of this theory, only two patients with transient monocular blindness associated with moving emboli in the retinal vessels have been reported in the literature (3, 4).

A frequent observation in patients with a history of amaurosis fugax is the

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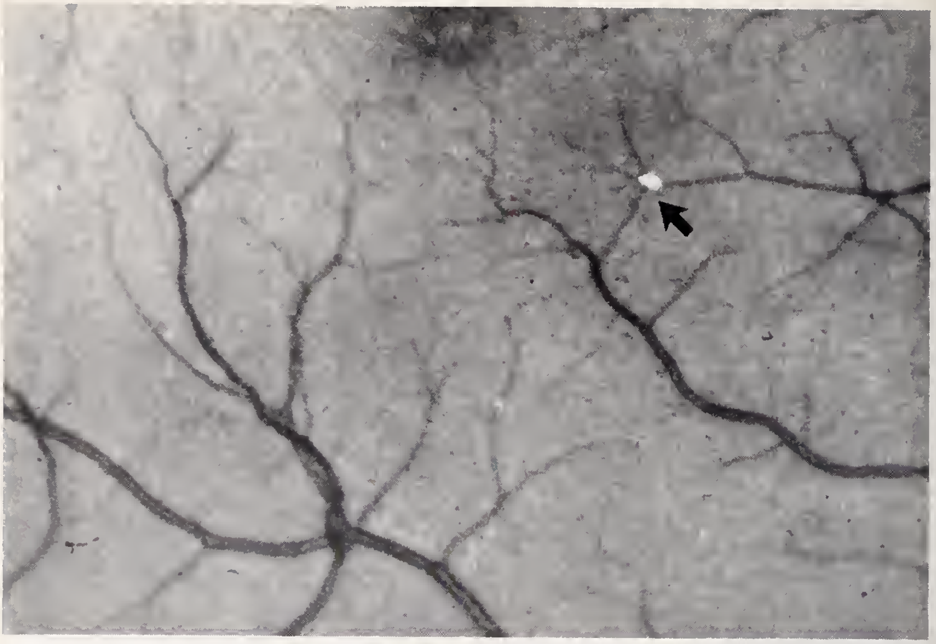

Fig. 1. Retinal photograph, showing yellow reflective particle (arrow) in lumen of retinal arteriole at bifurcation. No apparent obstruction of blood flow exists.

finding of shiny, reflective, yellow particles within the retinal artery lumen, usually at an arterial bifurcation. This material usually causes no obstruction of blood flow (Fig. 1). These bright plaques may occur in patients without a history of transient ischemic attacks. They should be carefully looked for in patients with histories suggestive of carotid stenosis. They are best demonstrated by looking at the bifurcations of the retinal arterioles while tilting the ophthalmoscope in order to reflect light off the bright plaque back into the observer's eye. This type of embolus is considered to be a cholesterol crystal. Less commonly seen are white, nonreflecting plugs of material within the lumen of the retinal vessels. These also have a predilection for being stopped at the bifurcation of the retinal arterioles (Fig. 2). Infarction of the retinal tissue is almost always associated with the "white plug" type of embolus (Fig. 3). It is this type of embolus which was seen moving along the retinal vessels in the course of observation of two patients during attacks of amaurosis fugax. Such emboli are considered to be composed of platelets, fibrin, and fats.

Evidence that such material seen within the retinal arterial lumens can arise from atheromatous plaques in the carotid is clinically supported by finding similar material within the retinal arterial lumens following carotid endarterectomies. Hollenhorst (1) found that 20 percent of the patients undergoing endarterectomy had such particles within their retinal vasculature. Similar observations have been made at The Mount Sinai Hospital on patients under-

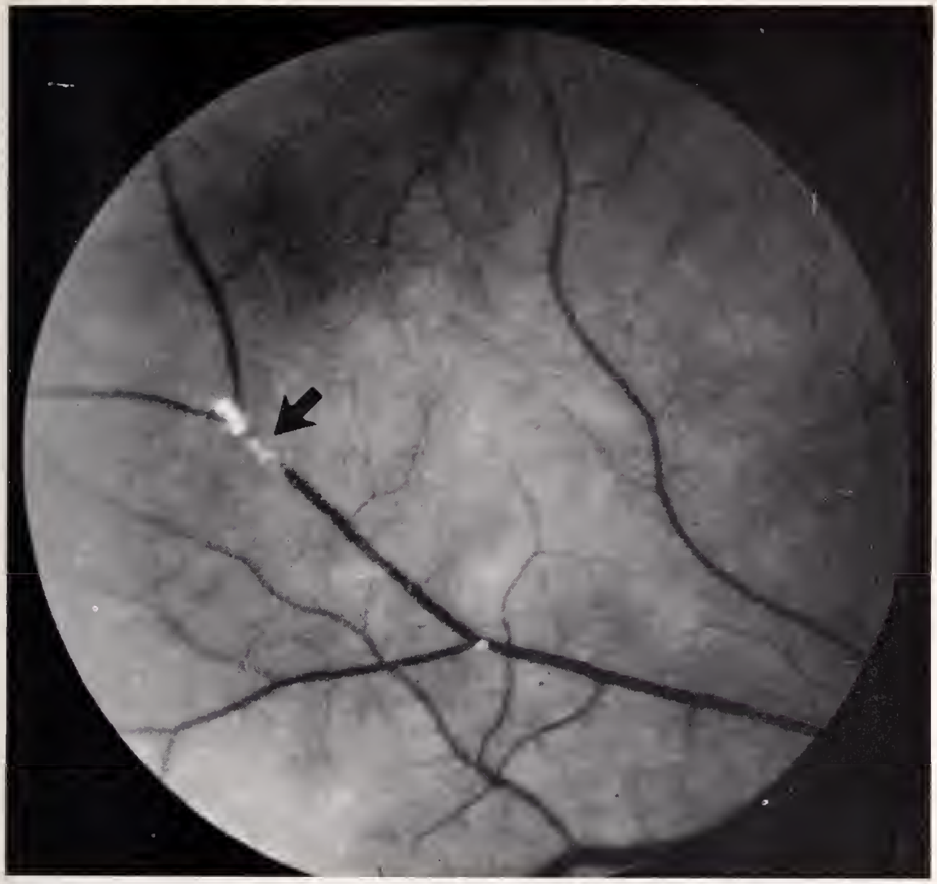

Fig. 2. "White plug" type particle (arrow) at bifurcation of retinal arteriole. The material seems to extend beyond the arteriolar wall, an appearance frequently observed.

going carotid endarterectomy. Other evidence suggestive of embolization is the cessation of transient ischemic attacks and the inability to observe intraluminal material after the carotid becomes completely occluded. Vision under such circumstances may remain excellent if the collateral circulation through the circle of Willis or the external carotid artery is adequate.

Transient ischemic attacks are much more frequent than permanent infarctions of the retina. Central retinal artery occlusion with complete loss of vision is probably related more to generalized arteriosclerotic disease than to atheromatous disease in the carotid. In a series of 84 retinal artery occlusions reported by Liversedge and Smith (5), only 2 were considered to be due to emboli. Reports have appeared very infrequently in the literature implicating embolization (6). The possibility of an association between carotid atheromatous disease and central retinal artery occlusion should not be ignored clinically.

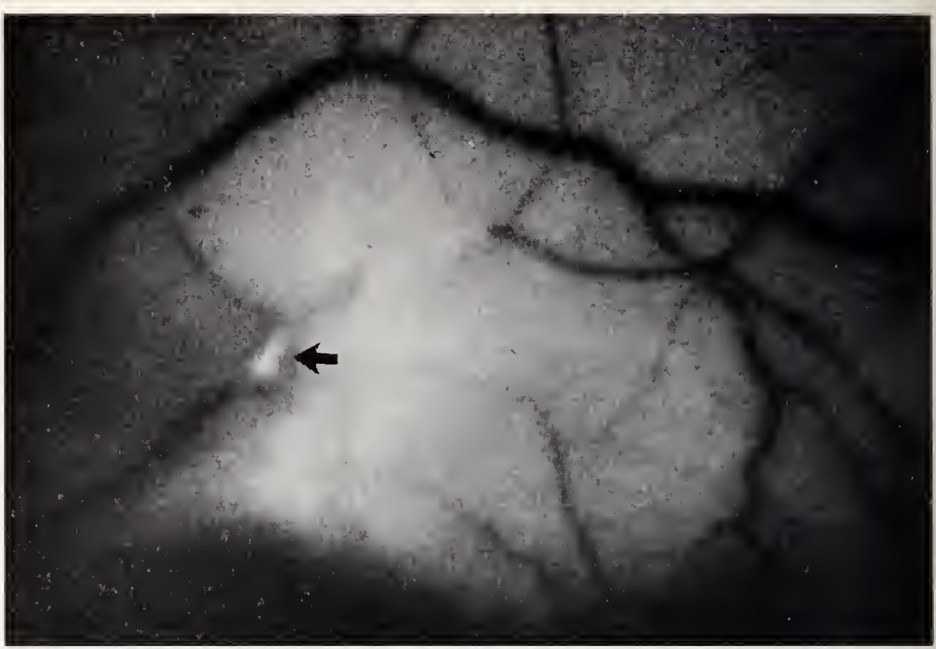

Fig. 3. A major retinal infarction is seen distal to the site of a "white plug" type particle (arrow) occluding an arteriolar lumen.

Transient loss of vision can occur with several conditions other than atheromatous disease. It has been seen preceding total loss of vision in cases of giant cell arteritis. It may also be secondary to embolization from valvular heart disease (rheumatic heart disease or subacute bacterial endocarditis). A case has been observed in fibrous dysplasia of the orbit. Brief obscurations of vision may occur in severe papilledema.

B. *Chronic Vascular Insufficiency.* Chronic vascular insufficiency to one or both eyes may be present in an eye with transient ischemic attacks (Fig. 4), or be preceded by episodes of amaurosis fugax. A stenotic lesion in, or an embolus to, the ophthalmic artery can produce vascular insufficiency in one eye. Bilateral occlusive disease in the neck may likewise cause unilateral or bilateral ocular vascular insufficiency because of inadequate collateral circulation. Nonatheromatous disease, such as Takayasu (pulseless) disease or syphilitic aortitis, may be responsible for similar ocular changes.

The eye with chronic vascular insufficiency may show conjunctival injection with corneal edema and folds in Descemet's membrane. The iris may be diffusely atrophic or show only focal areas of atrophy (Fig. 5). Posterior synechias bind the iris down to the lens creating an irregular immobile pupil. "Iritis" secondary to ischemia produces cells and a Tyndall effect in the aqueous humor of the anterior chamber of the eye. New vessels may develop on the iris surface and seal the chamber angle. The lens will become cataractous over several days

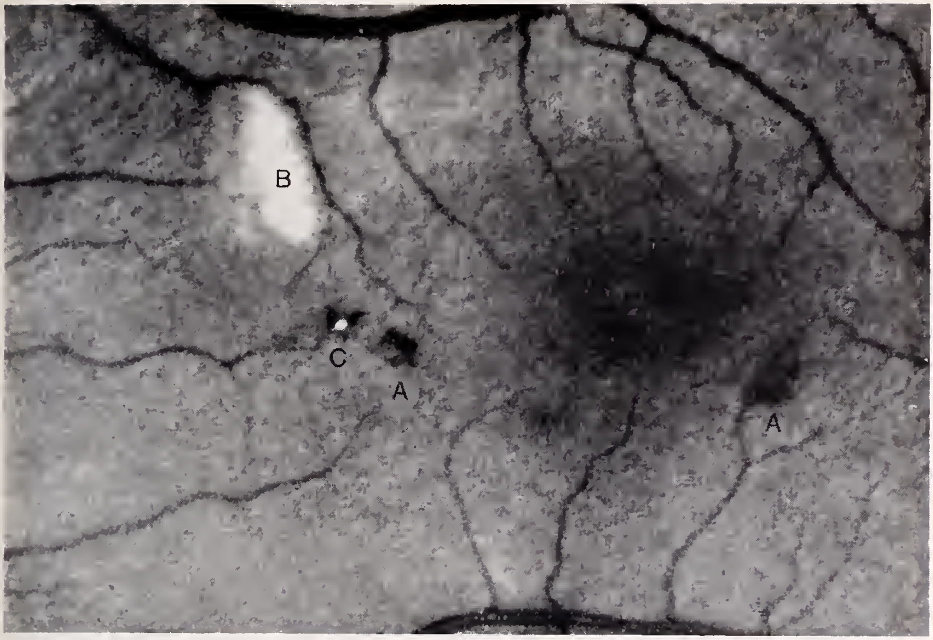

Fig. 4. Ocular fundus of patient with occlusive disease of internal carotid artery ipsilateral to eye photographed. Patient had attacks of amaurosis fugax. A = Areas of retinal hemorrhage due to chronic vascular insufficiency; B = "cotton wool" exudate (nerve fiber layer infarction); and C = bright reflective plaque in retinal arteriole.

if there is an abnormally slow turnover of aqueous humor, which is solely responsible for delivery of nutrients to, and removal of, waste products from the lens. Secretion of aqueous from the ciliary body can be severely depressed, as evidenced by the hypotony found in some eyes even in the face of a completely sealed chamber angle (the normal outflow channel for aqueous humor).

The retina in chronic vascular insufficiency shows many small hemorrhages. The arterioles appear narrowed with or without evidence of occlusion. The veins are dilated. Microaneurysms may be seen at the equator of the eye or at the posterior pole. Neovascularization occurs and is probably stimulated by the chronic hypoxia and blood stasis. "Cotton wool" exudates represent retinal nerve fibre layer infarctions. A swollen and pale optic nerve head is evidence of ischemia to the nerve (Fig. 6). Large areas of pale yellow color deep to the retina may represent choroidal infarctions. Choroidal infarctions are rare, because of an extensive overlapping anastomatic circulation. Eyes with severe vascular insufficiency have an extremely poor visual prognosis. This picture has been well described by Knox (7). It is uncommon in its extreme form, but lesser degrees are frequently overlooked (Fig. 4).

2. *Occlusive Disease of the Vertebral-Basilar Arterial System.* Transient ischemic attacks and permanent neurologic deficits occur with occlusive

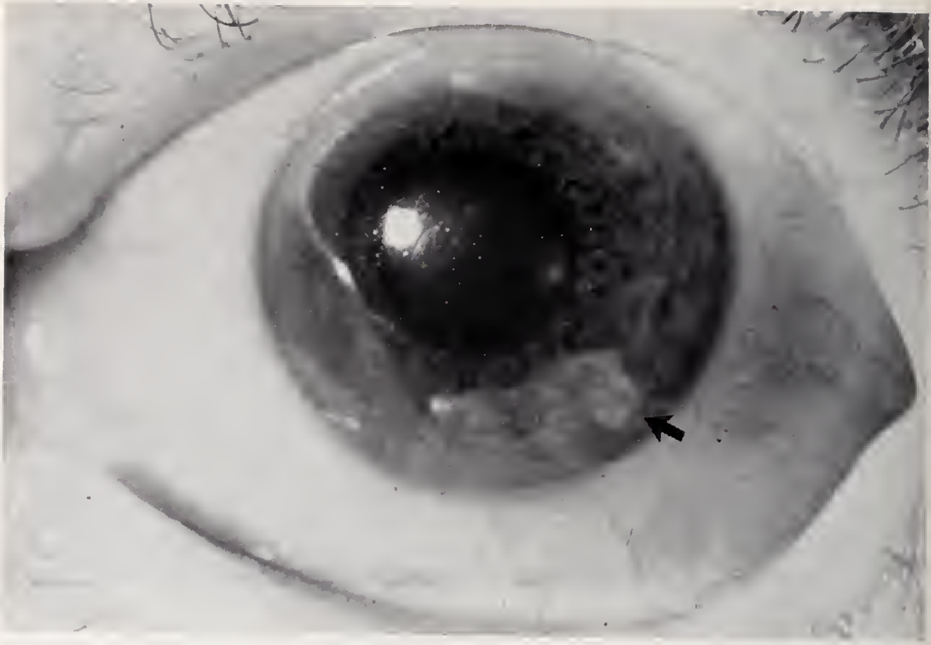

Fig. 5. Arrow points to focal areas of iris atrophy in patient with "iritis" due to ischemia. The ipsilateral carotid was occluded.

disease of the vertebral-basilar system. The ocular signs and symptoms are due to ischemia of the brain stem causing abnormal eye movements and pupillary responses; and ischemia of the posterior cerebral circulation affecting vision. Brain stem ischemia can cause double vision secondary to skew deviation, internuclear ophthalmoplegia, and/or possible involvement of cranial nerve nuclei. Nystagmus, vertigo, and a feeling of faintness are common symptoms. Ischemia in the distribution of the posterior cerebral arteries produces transient homonymous hemianopsia or complete cortical blindness with normal pupillary responses. Patients with such visual problems describe bilateral blurring of vision in both eyes or transient complete loss of vision.

3. Ophthalmic Diagnostic Tests for Carotid Disease:

A. *Ophthalmodynamometry.* Ophthalmodynamometry is a technique in which an instrument, as illustrated in Figure 7, is pressed against the temporal conjunctivoscleral coats of the eye after the instillation of a local anesthetic drop. The patient has no discomfort and feels only pressure being placed on the eye. As the instrument is pressed toward the center of the eye, the vessels on the optic disc are observed either with a direct or indirect ophthalmoscope. When the pressure of the ophthalmodynamometer against the globe increases the intraocular pressure above diastolic ophthalmic artery pressure, the central retinal artery completely collapses during diastole. The point at which this complete collapse of the vessel commences is taken as the diastolic reading. Pulsations of the retinal arteries continue until the intraocular pressure ex-

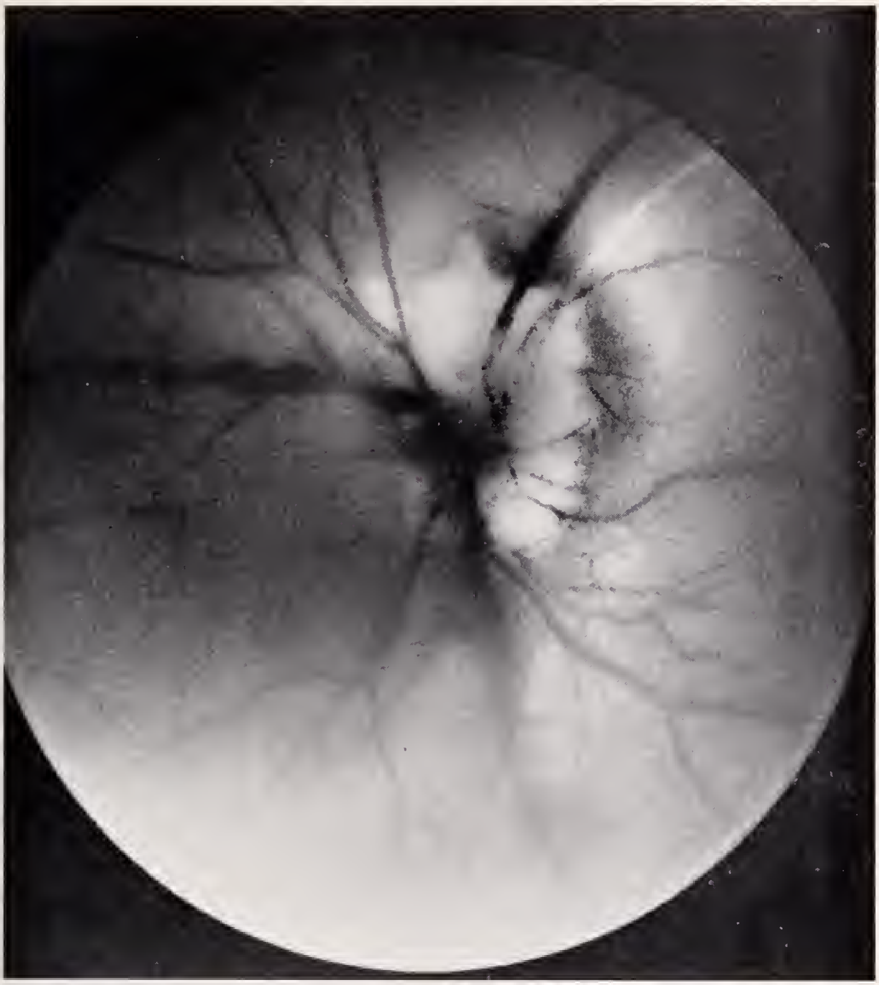

Fig. 6. Swollen pale sector of optic papilla and adjacent retina due to ischemia. Note dilated vein with hemorrhage along its borders (same patient as Fig. 5).

ceeds the systolic ophthalmic artery pressure. At this point all vascular pulsations cease and a systolic reading is taken.

The readings on the instrument are expressed in grams and are commonly reported in this form. Various tables and formulas have been devised to estimate ophthalmic artery pressure in terms of millimeters of mercury, but this conversion adds little to the interpretation of the data. The most significant finding is a difference in pressure between the two eyes. This difference may involve only the diastolic pressure, only the systolic pressure, or both measurements. A lowering of the diastolic or systolic pressure by 15 percent or more is considered significant in our hands. The paradoxical finding of a lower ophthalmodynamometric pressure on the supposedly uninvolved side is highly suspicious of bi-

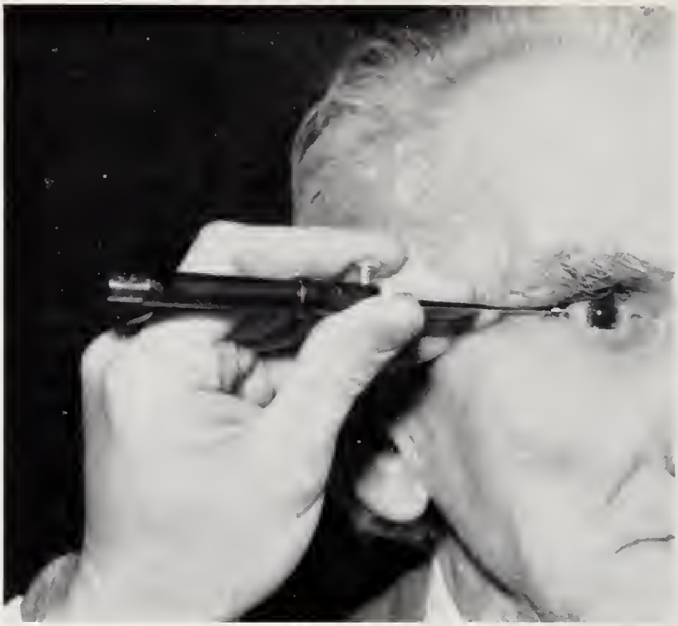

Fig. 7. The ophthalmodynamometer is shown being pressed against the patient's anesthetized eye.

lateral disease. Extremely low diastolic readings are not reliable and are not uncommonly found in normal individuals. Measurements taken in both the supine and standing positions may bring out differences in pressure between the two eyes which are not apparent with the patient in the usual sitting position. Compression of the contralateral carotid artery while taking the ophthalmodynamometric pressure may demonstrate a marked fall in ophthalmic artery pressure if little of the circulation is being supplied by the ipsilateral carotid. Cardiac arrythmias, or not taking into account large differences in the intraocular pressure, may lead to erroneous ophthalmic artery pressure readings. Blood pressures in both arms are taken at the time of examination.

New methods of ophthalmodynamometry are being developed using a perilimbal suction cup to increase the intraocular pressure, and obtaining a magnified view of the arteries on the optic disc by viewing through a contact lens (8). Such methods may eventually increase the accuracy of this technique.

B. *Ophthalmodynamography (9).* Ophthalmodynamography is a technique in which an air-filled balloon is strapped, by means of goggles, tightly against the orbit. The patient keeps both eyes closed. The pulsations from the eye and structures in the orbit are transmitted to the balloon and recorded. The pressure within the balloon can be increased, which in turn increases the pressure against the orbital structures. As the pressure against the orbital structures is altered, a change in the calibre of the transmitted pulsations occurs. A diastolic and systolic ophthalmic pressure can be recorded by this means.

C. *Oculosphygmography* (*10*). Oculosphygmography relies on the fact that with a high enough degree of carotid stenosis, less blood flow will be pushed into the eye during each systole, and the pulse pressure, more specifically the pulse-synchronous variations in the intraocular pressure, will be reduced on the side of the carotid occlusion. The extent of this pulse pressure is measured by placing a 20 gram weight on the anesthetized cornea. A sensitive piezoelectric crystal measures the amplitude of the pulsations. Similar observations on the difference in the pulse wave between the involved and uninvolved sides can be made with an ordinary sensitive tonometer routinely used for measuring intraocular pressure.

D. *Tonography as Used for Diagnosing Carotid Occlusive Disease* (*11, 12*). Tonography is a technique used to measure the ability of aqueous humor to leave the eye. A pressure measuring device of a given weight (a tonometer) is placed on the anesthetized eye for a period of 4 minutes. If the normal exit pathways of aqueous humor are blocked, no aqueous can be "pressed" out of the eye, and the pressure at the beginning and at the end of 4 minutes will almost be identical. If, however, the pathways for aqueous outflow are open, a great deal of fluid can be pushed out of the eye, and there will be a significant fall in pressure over the 4 minutes. This pressure fall can be recorded continuously while the tonometer is placed on the anesthetized cornea. If during tonography the ipsilateral carotid artery is compressed, there will be an immediate slight drop in intraocular pressure which is probably secondary to a decrease in the blood volume delivered to the uveal coats of the eye. When pressure on the carotid is released, the intraocular pressure immediately returns to normal. Compression of the contralateral carotid under normal circumstances produces no change in the intraocular pressure. If one carotid is partially occluded or totally occluded, compression of this artery will have little or no effect on the intraocular pressure of either eye. However, compression of the uninvolved carotid will produce a large decrease in intraocular pressure of both the ipsilateral and contralateral eye, especially if little or no other collateral supply to either eye is present.

E. *Fluorescein Angiography* (*13, 14*). Fluorescein may be injected into an antecubital vein and observed passing through the ocular vascular system. The fundus is observed with a blue light which activates the fluorescein to fluoresce in the green. An attempt is made to inject the dye as a bolus into the antecubital vein. The bolus travels to the right side of the heart, pulmonary system, left heart, aorta, and eventually into the ophthalmic artery through the internal carotid system. An attempt can be made to time the lapse between the injection time and the appearance of the dye at the retinal arteries on the optic disc. Because of the variability in the injection procedure, a variety of circulation times can be arrived at by this method. If the fluorescein is given and both eyes are observed, a discrepancy in the arrival time of the fluorescein at the retinal arteries in each eye can be determined. In one study (3), the normal "arm to retinal" time was 11 to 13 seconds. The difference between the two eyes was approximately 1 second. In carotid occlusive disease, this difference ranged between 1.0 to 9.2 seconds. This type of study requires good patient cooperation, two competent observers, and one person for injecting and keeping time.

F. *Thermography.* A variety of methods have been used to delineate a lower temperature about the orbit ipsilateral to an involved carotid. If there is a true decrease in blood flow, the skin about the orbit on the involved side (supplied by the terminal branches of the ophthalmic artery) may be of a lower temperature than on the uninvolved side.

G. *Auscultation.* Auscultation of the carotids should always be performed. A bruit is a highly significant finding. At times, it may be contralateral to the side of the complete occlusion due to increased blood turbulence. Transmission of a heart murmur to the neck vessels must be excluded. Palpation of the carotid may be useful if a pulse difference exists.

Most of the tests outlined above are highly significant in complete occlusive disease of the carotid or in high stenoses. Lesser degrees of stenosis go undetected by any of these methods. In ophthalmodynamometry it is known that the pressure head in the ophthalmic artery will not decrease significantly until the carotid has been occluded to approximately 85 percent. This is clearly demonstrated in patients who are followed with ophthalmodynamometry while a carotid is being slowly closed by means of a screw clamp. A drop in the ophthalmic artery pressure may not be seen until the final turn of the screw is made which occludes the carotid.

4. Diagnostic Tests Involved in Vertebral-Basilar Insufficiency. The ophthalmologist has less to offer in diagnostic testing in Vertebral-basilar insufficiency. Visual fields are important in substantiating field loss. A difference in brachial artery pressure, asynchronous radial artery pulses, diminished or absent pulses in one arm, and/or a bruit over the supraclavicular area may indicate a subclavian steal syndrome. In this syndrome there may be an atherosclerotic block proximal to the take-off of the vertebral artery. When the arm on the involved side is exercised, a vascular insufficiency in the arm may develop. Blood from the contralateral vertebral artery, instead of going into the basilar artery, may flow down the opposite vertebral artery into the arm with vascular insufficiency. This deprives the brain stem of its normal complement of blood and may lead to a transient ischemic episode.

Summary

This article has reviewed how the eye and visual systems can be affected by occlusive arterial disease in the neck. A number of diagnostic tests have been outlined which may be used by the ophthalmologist in the work-up of these patients. These procedures are frequently a useful supplement to the neurologist's examinations.

Acknowledgments

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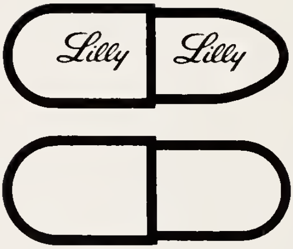

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Alexander B. Gutman

SOLOMON SILVER, M.D.

It is not easy in the space of a few pages to create a picture of a man whom you have known for some 50 years, first as a youth in high school in what is now the severely troubled area of Bedford-Stuyvesant. At that time Boys' High School had as its major student body sons of first generation lower middle class European immigrants who, with their parents blessings and real sacrifices, furnished an astounding number of distinguished leaders in the arts and sciences. Not the least of these is the recipient of the tribute of this volume.

Al distinguished himself both as a student and leader in school activities even at that time. His background was more favorable than that of most of us because his father was a respected physician. This probably conditioned his choice of medicine as a career, and he was prescient enough at that time (1920) to realize that a strong basis in science would be the cornerstone of medicine as it was developing. By combining studies at Cornell and at the University of Vienna he was able to earn the degrees of Ph.D. (Cornell, 1926) and M.D. (Vienna, 1928). He anticipated by some 50 years the "new" programs that some medical schools are offering today.

Since 1929 he has devoted "full time" to teaching and research and has achieved world-wide acclaim in both fields. Recognition did not come easily nor early, but by 1945 he was an Associate Attending Physician at Presbyterian Hospital and in 1949 was appointed Professor of Medicine at Columbia-Presbyterian Medical Center. From 1947 to 1951 he served as Director of the Columbia Research Service at Goldwater Memorial Hospital where he created a clinical service oriented toward metabolic research that was unique in the city hospital complex. In 1951 The Mount Sinai Hospital decided that its needs in the Department of Medicine could only be served by a full-time Director and the position was offered to Dr. Gutman, which he held with distinction until his voluntary retirement in 1968. By this time, and largely through his efforts, The Mount Sinai School of Medicine was a reality, and it is a measure of the man to know that he retired before such retirement was compulsory so that his successor would have a free hand in organizing the Department of Medicine in the new school. I know that this entailed a great personal sacrifice because he always looked forward to being the executive head of the Department of Medicine in the School, but he was willing to forego that ambition in order to leave a free field for his worthy successor, Dr. Solomon Berson.

His educational activities extended far beyond the confines of the institutions where he worked. As Associate Editor of the *Cecil-Loeb Textbook of Medicine* from 1950 to 1960, he played a large role in the development of medical students throughout the world. In 1946, single-handedly and against apparently insuperable odds, he founded and edited The American Journal of Medicine. This is probably one of the most respected publications in internal medicine in the world today, and its high standards are entirely due to the exacting demands of its Editor. Physicians all over the world consider it a privilege to publish their studies in the "Green Journal" and Dr. Gutman has never compromised its high standards. The Journal has no "captive audience" and its long list of voluntary subscribers is almost unique in medical publications.

His scientific collaborator of many years, Dr. Yu, will record some of the scientific contributions made by Dr. Gutman, but I cannot close without an expression of my deep affection and respect for a real leader in American medicine, a sincere friend, and a contributor to the greater glory of The Mount Sinai Hospital and Medical School.

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Publications: Approximately 250 papers, in recent years chiefly on gout, purine metabolism, renal physiology. One book, *Modern Drug Encyclopedia*, Yorke Publishing Co., New York 1946, 3rd Ed., 1157 pp.

The Scientific Achievements of Dr. Alexander B. Gutman

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While still a school boy, Dr. Alexander B. Gutman had great interest in chemistry, making use of the facilities of his father's chemistry laboratory. His interest in chemistry was further developed at Cornell University, where he majored in Experimental Biology and received his B.A., M.A., and Ph.D. degrees. He received his M.D. degree at the University of Vienna. It is indeed remarkable that he managed to earn four degrees with honors in 8 condensed years.

His initial research interest was in the thyroid gland, especially the relationship to amphibian metamorphosis. His Ph.D. thesis was on the effect of thyroxin and diiodotyrosine in amphibian metamorphosis. Later, at the College of Physicians and Surgeons, Columbia University, he reported the first analyses of thyroxine in the human thyroid gland, and made significant contributions to the study of hyperthyroidism. This led to interest in the adjoining parathyroid glands. From these, he studied various metabolic disorders of bone, and the enzyme alkaline phosphatase. His investigation of serum alkaline phosphatase in liver disease with Dr. Franklin Hanger led to the discovery of drug induced intrahepatic cholestasis.

Turning to acid phosphatase, Dr. Gutman, in collaboration with his late wife, Ethel Benedict Gutman, discovered the increased levels of serum acid phosphatase in metastatic prostatic carcinoma, for which he was awarded the Francis Amory Prize in 1948 and the Valentine Award in 1967.

In the early 1940's, his interest shifted to serum proteins. Pioneering studies combining chemical, electrophoretic, and immunological methods on serum proteins in multiple myeloma led to the identification of Bence Jones protein in the serum and the introduction of the term M protein in multiple myeloma. This work was a forerunner of the present day studies on the multiple myeloma proteins, a cornerstone laid more than a quarter of a century ago.

In the late 1940's Dr. Gutman became interested in gout, which has become his principal research activity since then. As the Director of the Department of Medicine of The Mount Sinai Hospital, although busy with various administrative, teaching, and clinical responsibilities, he managed to pursue the study of gout without interruption.

In exploring the nature of the metabolic errors of primary gout, it was found that in a number of such patients, overproduction of uric acid was demonstrable by the use of the labelled precursor of uric acid, glycine-^{15}N. Even in those with normal incorporation, there was undue enrichment of uric acid-N at positions 9 and 3, both derived from glutamine. The increased enrichment of uric acid-N at positions 9 and 3, together with the defective renal production

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of NH_3, which is also derived from the same substrate glutamine, led to the proposition that in gout there must be a diversion of glutamine from renal production of NH_3 to de novo uric acid biosynthesis.

Through the observation of paradoxical action of salicylate upon the renal excretion of uric acid, the bidirectional transport mechanism of uric acid excretion was proposed and studied. Simultaneous tubular reabsorption and secretion of uric acid were demonstrated, and the relative magnitudes of tubular secretion were studied in man, dog, and chicken.

Dr. Gutman introduced protracted uricosuric therapy, leading to the disappearance of tophi and the prevention of their development. This work ushered in a new era of management of patients with gout.

In recognition of his contribution in elucidating the metabolic defect in gout and the clinical application to its management, he received the Gairdner Foundation Award in 1961, and the Holbrook Memorial Lectureship Award, Arthritis and Rheumatism Foundation, in 1964. On the occasion of the 200th anniversary of the founding of the Medical School of King's College, the College of Physicians and Surgeons, Columbia University, he was given the Distinguished Achievement Award in 1967.

While history in science was being made in his laboratory, Dr. Gutman sprouted in other directions as well. The "Green Journal" was born in 1946. This baby of his flourished by leaps and bounds, and it is widely read by the medical profession. Every article is carefully chosen, edited, and proof-read in person, and he received the Distinguished Service Award in Medical Journalism in 1953.

He was the Associate Editor of the *Cecil-Loeb Textbook of Medicine* from 1950 to 1960. During this 10 year period, three editions were published. Needless to say, a lot of candlepower was spent in reading and editing this famous textbook, universally read by all medical students.

With his profound knowledge of metabolic diseases, he has been closely involved in policy making regarding medical research activities sponsored by the National Institutes of Health of the U.S. Public Health Service. He was a member, then Chairman of the Metabolism and Nutrition Study Section (1953–58), on the Board of Scientific Counselors (1960–64), and a member of the National Advisory Arthritis and Metabolic Diseases Council, USPHS (1964–68). For years, prior to each study section or Council Meeting in Bethesda, he reviewed all project applications thoroughly so that no worthy project would be passed without receiving appropriate attention.

As President of the Harvey Society for the years of 1963 to 1964, he spent much time in writing to the most eminent speakers, and studied each speaker's work so carefully that his introduction practically covered the essence of the speaker's life work. In 1964, he gave a most impressive and illustrious talk on the biological significance of uric acid at the Harvey Society.

As mentioned by Dr. Solomon Silver, after much effort in bringing Mount Sinai School of Medicine into reality, he retired as Chief of Medicine in 1968.

Since many of his responsibilities in administration have been relinquished, his devotion and interest in research have intensified. He is as busy as ever, the light at the desk continues to glow long hours each day, and, of course, the "Green Journal" keeps on expanding and growing.

As his close associate since 1947, it is my privilege to present Dr. Alexander B. Gutman as a distinguished scientist, a man with decades of scientific activity in many spheres. The curriculum vitae which follows Dr. Silver's paper and the bibliography which follows this appreciation will speak for themselves.

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Effect of Trypsin and Phytohemagglutinin on Template Capacity of Mammalian Nuclei for RNA Polymerase*

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The small, peripheral blood lymphocyte is considered to be a resting cell, with a low mitotic rate *in vitro* (1–3), and a prolonged life span *in vivo* (4). However, under the influence of several mitogens, such as phytohemagglutinin (PHA) (5, 6), lymphocytes increase their synthesis of RNA (7) and protein (8), replicate DNA (9), and divide. These biochemical changes are also expressed morphologically. Thus, dense, inactive heterochromatin becomes fibrillar, metabolically (10) active euchromatin, nucleoli appear, the cytoplasm enlarges and acquires pyroninophilia and basophilia (11), while increases in the activity of several enzymes (12–14) can be demonstrated histochemically. These events culminate in mitosis. During this process, termed "stimulation" or "transformation," lymphocytes synthesize at least one protein (interferon) (15) not previously detectable, and augment considerably their content of organelles (16, 17), enzymes (12–14, 18, 19), and secretory proteins (20). Thus, the stimulation of small lymphocytes by PHA has been considered a model of widespread gene activation in mammalian cells.

However, no new messenger RNA has been shown to be transcribed from the previously inactive DNA of resting lymphocytes. Hybridization and competition studies of the RNA produced by the transforming cell have not demonstrated sequences significantly different from those in the resting cell (21). Moreover, increased amounts of DNA-like RNA in the stimulated cells have not been shown by base-pair analysis (22). But the absence of this sort of definitive evidence does not exclude changes at the transcription level, since the sensitivity and specificity of such methods have not been established in mammalian systems (wherein DNA redundancy is crucial). Furthermore, the early shifts from dense heterochromatin to almost exclusive euchromatin, associated with increments in acetylation of histones and turnover of nuclear phosphoproteins, have been taken to indicate changes in controls of transcription (23, 24).

Direct evidence of transcriptional controls might be obtained from studies of the template activity of nuclei from control and PHA-stimulated cells. The capacity of isolated nuclei or chromatin to act as template for RNA synthesis in the presence of exogenous RNA polymerase is one indicator of the amount of the DNA available for transcription in the cell (25). Therefore, to

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determine if lymphocyte stimulation is associated with changes in the availability of DNA, we have examined the capacity of nuclei from resting and stimulated lymphocytes to prime for RNA synthesis, and have also studied their response to trypsin. The template activity of mammalian cell nuclei and chromatin has been shown to be increased by the *in vitro* action of trypsin (26, 27). We have previously shown that very shortly (2 hours) after stimulation with PHA or pokeweed mitogen, there may be found, in cell homogenates, a redistribution of acid hydrolases from granular to unsedimentable fractions of the cell (28, 29). It appeared possible that this redistribution of enzymes might expose nuclei to a previously latent or inactive hydrolytic enzyme, the action of which might grossly resemble that of trypsin. Therefore, experiments were first performed in which conditions were established for the study of trypsin-induced increments in template capacity for RNA polymerase.

Template Capacity of Rabbit Liver Nuclei For RNA Polymerase and the Effects of Trypsin

Since rabbit liver constitutes a more abundant source of nuclei than human lymphocytes, this tissue was used to establish conditions under which template activity could be measured, and the effects of trypsin studied. Rabbits (approximately 1 Kg were exsanguinated by rapid withdrawal of 60–70 ml of blood followed by air embolus. The bloodless liver was removed, minced, and repeatedly washed in 0.25 M sucrose containing 0.05 M tris buffer (pH 7.5) with 0.025 M KCl and 0.005 M $MgCl_2$ (= sucrose/TKM), at 4°C. Minced liver was homogenized in 10 ml aliquots of a 1:3 (w/v) suspension in 0.25 M sucrose TKM with 10 strokes of a teflon/glass motor-driven homogenizer (Tri-R-Instruments, Jamaica, N.Y.). The homogenate was filtered through 3–4 layers of wire mesh to remove debris and 0.25 M sucrose/TKM was added to yield a final 1:6 (w/v) suspension. The suspension was centrifuged for 10 minutes at 2,500 rpm in a Servall SS-3 centrifuge. The resultant pellets were resuspended to 40 ml with 0.88 M sucrose/TKM to which 120 ml of 2.64 M sucrose/TKM were added to yield 2.2 M sucrose/TKM. After centrifugation for 60 minutes at 22,500 rpm in the SW 25 rotor of a Spino L-2 preparative ultracentrifuge, the nuclear pellets were resuspended in 50 ml of 0.88 M sucrose/TKM. The nuclei were again centrifuged in a Servall SS-3 centrifuge for 10 minutes at 2,500 rpm, and finally resuspended in 1–2 ml of 0.88 M sucrose/TKM. All procedures were carried out at 4°C. DNA and protein were determined by the standard Burton and Lowry procedures, and nuclei were diluted to appropriate final DNA concentrations as indicated below.

Nuclei to be treated with typsin were exposed to 3 X recrystallized enzyme (Worthington) for 30 minutes at 37°C at pH 7.5 (in 0.2 M tris/HCl buffer). After incubation, soybean trypsin inhibitor (Worthington) was added (2:1, w/w, inhibitor:enzyme) for 10 minutes at 37°C, and the addition of inhibitor to appropriate controls showed no inhibition of the RNA-polymerase assay. Template capacity for RNA polymerase (*M. lysodeikticus*, Miles Labora-

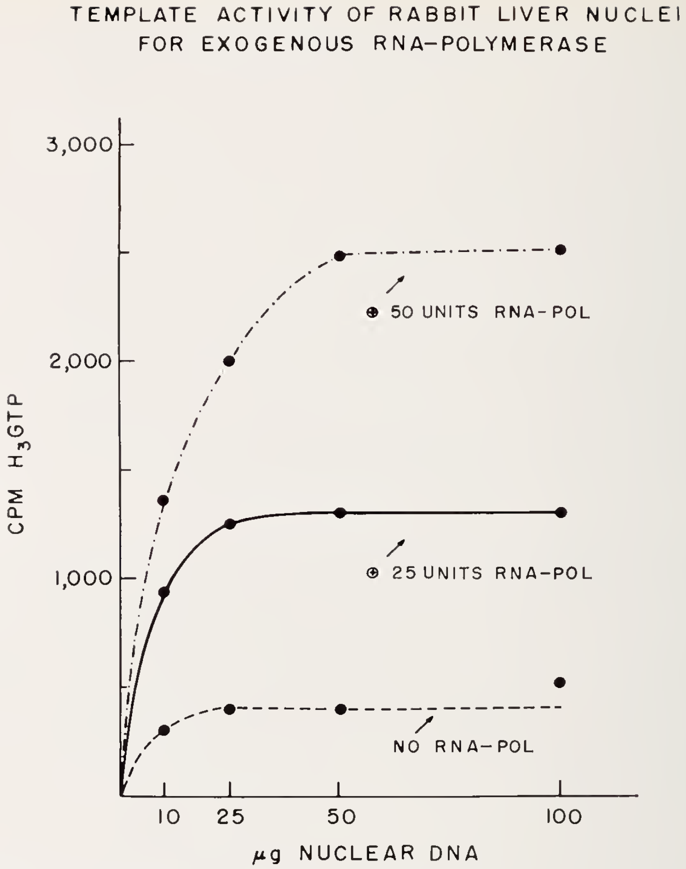

FIG. 1. Effect of increasing amounts of RNA polymerase (*M. lysodeikticus*) on the template activity of rabbit liver nuclei (see text for details).

tories) was assayed by methods described in detail below. The results shown in Figure 1 established that at 50 units of RNA polymerase/assay, 5–25 micrograms of DNA/assay were in the range where only the amount of DNA was limiting to the reaction. The studies indicated that the assay could be used to determine the amount of DNA available for transcription by added polymerase, since addition of equal amounts of enzyme yielded increments of RNA synthesis proportional to the amount of DNA present. Using 20 μg of nuclear DNA, and incubating nuclei with increasing amounts of trypsin (Fig. 2), it was shown that trypsin treatment rendered the nuclei capable of acting as better templates for RNA synthesis. Points at the ordinate represent the template activity of nuclei *not* treated with trypsin. These experiments document that when nuclei are treated with a defined hydrolytic enzyme, their DNA is rendered more available for template function in the presence of added bacterial enzyme.

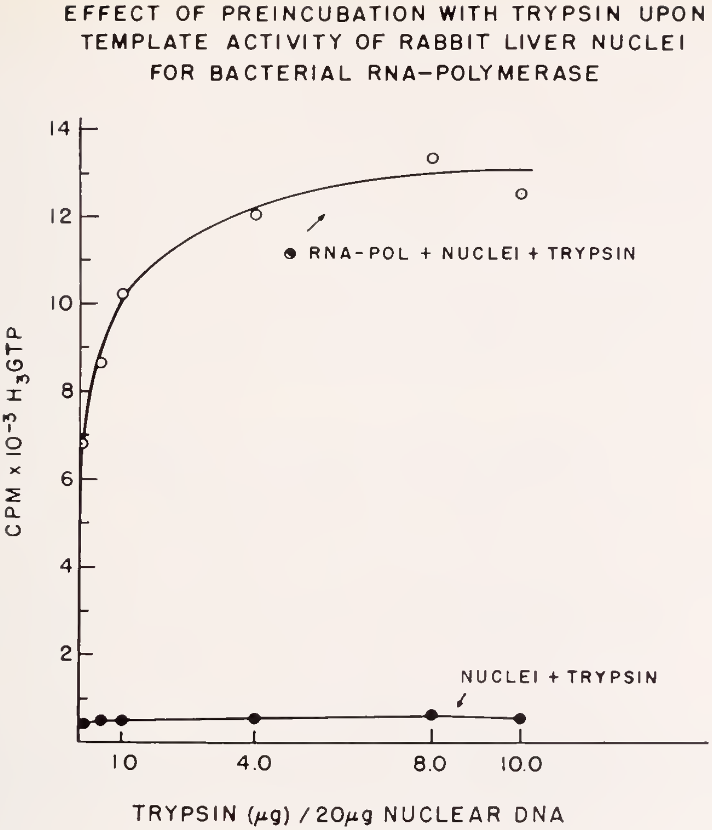

FIG. 2. Effect of preincubation with trypsin upon template activity of rabbit liver nuclei for bacterial RNA polymerase. Points at ordinate represent nuclei *not* treated with trypsin. ⊙——⊙ = in presence of added RNA polymerase, ●——● in absence of added RNA polymerase.

Template Capacity of Human Lymphocytes for RNA Polymerase

Lymphocytes were isolated and purified from human peripheral blood and cultured as previously described (30), but at the slightly lower cell concentration of 50–70 × 10^6 cells per 40 ml culture media per tube. Each batch lot of PHA-P (Difco) was assayed for mitogenic potency as determined by the capacity of various concentrations to stimulate C^{14} thymidine incorporation into DNA 24–48 hours following addition. A dose in the optimal range was then used. Cultured cells were harvested at the indicated time intervals as previously described (30).

The washed cells (5–7 × 10^7) were suspended in 4 ml of 0.34 M sucrose and homogenized in a Bellco Plexiglass homogenizer (#1980) with 10 up and down strokes of the shaft, which was driven by a variable speed motor (Tri-R-Instruments, Jamaica, N.Y.) at speed setting 5. The homogenates from two tubes were transferred to 50 ml lusteroid tubes, the homogenizer washed with an ad-

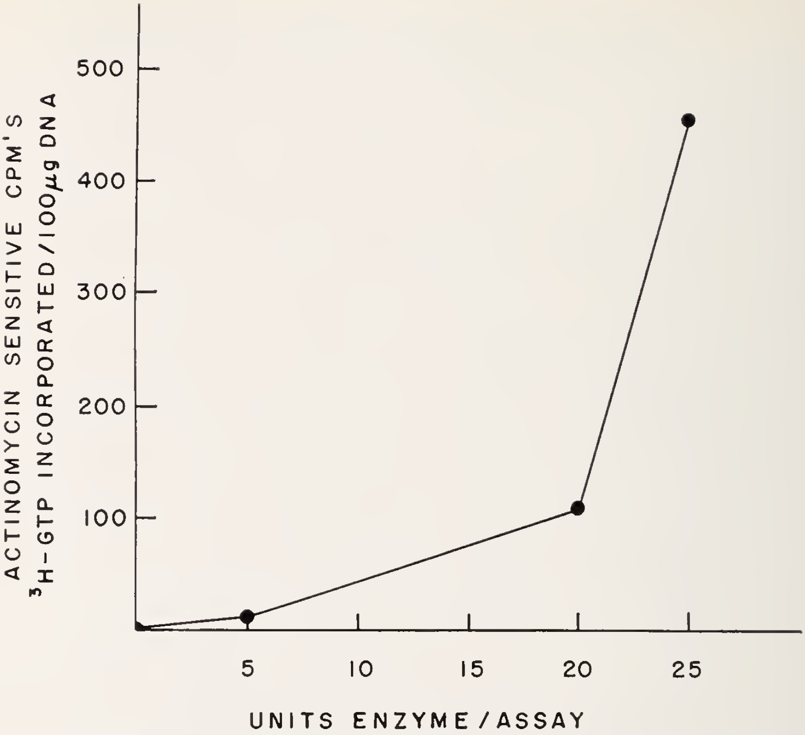

FIG. 3. Effect of RNA polymerase concentration upon the incorporation of H_3GTP into RNA by isolated nuclei: Nuclei were isolated and incubated without RNA polymerase and in the presence of 5, 20, and 25 units of exogenous RNA polymerase (*M. lysodeikticus*).

ditional 4 ml of sucrose, and the suspension centrifuged in a Servall Superspeed Model SS-3 at 800 g × 10 minutes. The supernatant was decanted and the nuclear pellet resuspended in 2–3 ml of 0.34 M sucrose, 0.05 M tris (pH 7.5), .005 M $MgCl_2$. Disruption of cells was monitored by observation of crystal violet-stained wet preparations under dark field illumination. Only occasional non-disrupted cells were seen. The DNA concentration was determined by the method of Burton (31), protein by the method of Lowry et al (32), and RNA (33) by the orcinol method.

The nuclear isolation procedure yielded only 30–33% of the DNA content of the whole cells. However, several criteria would suggest that there was no differential loss between stimulated and unstimulated cells. The protein:DNA ratio was not significantly different, averaging ($n = 4$) 7.3 ± 0.9 for nuclei from unstimulated cells and 8.0 ± 2.3 for nuclei from stimulated cells. This compares to a protein:DNA ratio of 4.8 reported for liver nuclei isolated by the method of Widnell and Tata (34). The RNA:DNA ratio was 0.32 for control and 0.29 for stimulated lymphocyte nuclei, compared to 0.22 in liver nuclei. The isolated nuclei were assayed in a mixture (0.5 ml) containing 58 μmoles (35) tris buffer (pH 7.5), 1.2 μmoles spermidine HCl, 1.42 μmoles

$MnSO_4$, 1 μmole $MgCl_2$, .450 μmoles each of nucleotide triphosphates (ATP Na_2, CTP Li_4, GTP Li_4, and UTP Li_4), 3.33 μcuries H_3GTP (~3 mμmoles), 2–50 μg nuclear DNA, and 50 U RNA polymerase (*M. lysodeikticus*, Miles Laboratories). All assays were performed in duplicate, and usually at three concentrations of DNA/assay for each experiment. Other aliquots of the reaction mixture were assayed in the presence of 25 μg Actinomycin D (Lyovac Cosmogen®, Merck Sharpe and Dohme) and all results are reported as: CPM incorporated in the absence of Actinomycin D minus CPM incorporation in the presence of Actinomycin D (Actinomycin-sensitive CPM's). Under these conditions of assay, the level of endogenous RNA polymerase was insufficient to cause incorporation of H_3GTP into RNA. Indeed, incorporation was not detected until 20–25 U of exogenous RNA polymerase enzyme were added per assay (Fig. 3).

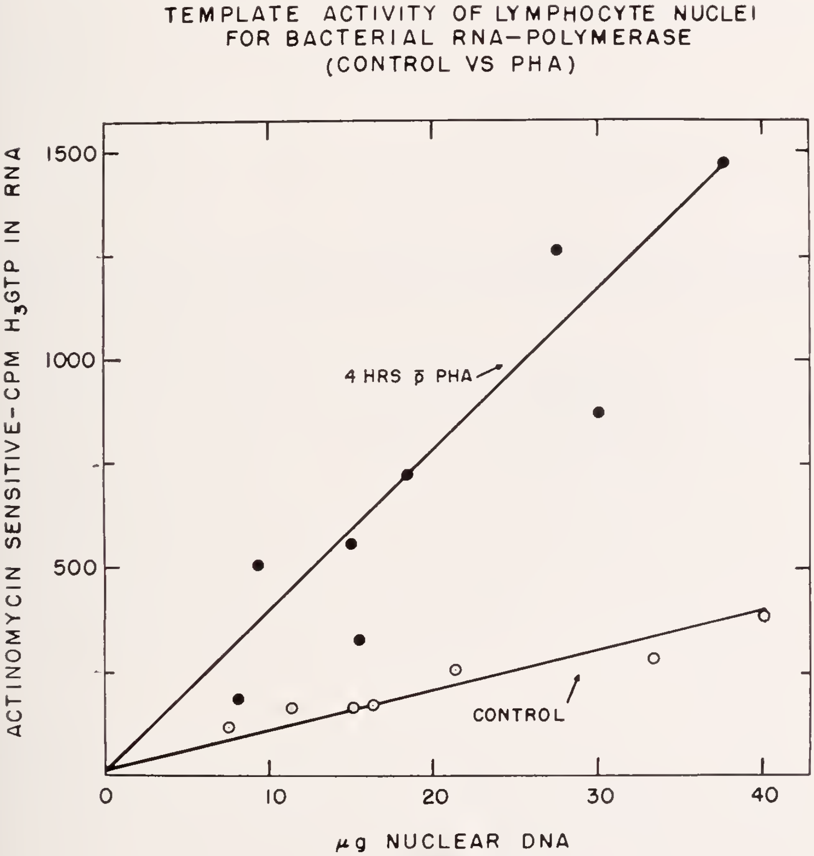

FIG. 4. Effect of stimulation of lymphocytes with phytohemagglutinin for 4 hours upon template capacity of the isolated nuclei: Lymphocytes were incubated for 4 hours with and without PHA; nuclei were isolated and incubated for 10′ at 37°C in the presence of 50 U RNA polymerase (*M. lysodeikticus*) and H_3GTP incorporation determined as in Figure 1. Each value is the average of duplicate determinations of CPM incorporated in the absence of Actinomycin D minus the CPM incorporated in the presence of Actinomycin D.

TABLE I

*Effect of Freezing and Thawing upon Template Capacity of Nuclei Isolated from Human Lymphocytes**

Nuclei	No. of Expts.	Control ± SEM	PHA ± SEM
Intact	5	500 ± 37.0	2,040 ± 150
Disrupted	5	430 ± 13.0	1,760 ± 122

* Lymphocytes were incubated for 2 hours with and without PHA. Aliquots of the isolated nuclei were rapidly frozen and thawed six times and the nuclei assayed for their capacity to prime for Actinomycin D-sensitive-H_3GTP incorporation into RNA in the presence of exogenous RNA polymerase (assay conditions as described in the text). Values represent Actinomycin sensitive CPM ^{14}C-GTP incorporated into RNA/100 μg DNA ± SEM.

Assays were incubated for 10′ at 37°C and the reaction was stopped by the addition of 5 ml of ice-cold 0.2 M perchloric acid in 0.02 M $Na_4P_2O_7$. The reaction mixture was filtered onto Millipore filters (HAWP 02400, HA 0.45 μ) and washed four times with 4 ml and four times with 1 ml of ice-cold 0.04 M perchloric acid in .004 M $Na_2P_2O_7$. The filters were air dried and placed in vials with toluene POP, POPOP, and counted in a Packard Tri-Carb scintillation counter (Model 500 B) with 25 percent efficiency for tritium. For all other experiments, 50 units of enzyme were added per assay so that the level of RNA polymerase added was sufficient to allow for a linear response with increasing DNA/concentration (Fig. 4). The incorporation was linear for at least 10 minutes.

Effect of Stimulation of Lymphocytes with PHA upon Template Capacity of the Isolated Nuclei

In order to determine if there was a change in template capacity, lymphocytes were incubated with and without PHA for 4 hours, the nuclei isolated, and their ability to prime for the incorporation of H_3GTP into RNA was determined in the presence of added RNA polymerase. Four hours after stimulation (Fig. 4), nuclei isolated from stimulated cells primed for the production of greater amounts of RNA than nuclei from control cells. This was true at all concentrations of DNA per assay tested, the average increase being threefold (3.1 ± 0.21).

This increase in template activity was observed even at 2 hours after stimulation (Table I). To determine if this increased incorporation of H_3GTP into RNA might reflect a differential permeability of the nuclear membrane to nucleotides or enzyme, aliquots of the nuclear preparations were rapidly frozen in dry ice-acetone and thawed at 37°C six times. Control nuclei (Table I) incorporated 500 ± 37 CPM/100 μg DNA compared to 430 ± 13 CPM/100 μg DNA after disruption. Nuclei from PHA-stimulated cells incorporated approximately four times as much H_3GTP into RNA whether intact (2,004 ± 150 CPM/100 μg DNA) or disrupted (1,760 ± 122 CPM/100 μg DNA). In addition, in one experiment, nuclei treated with Triton X-100 prior to freezing

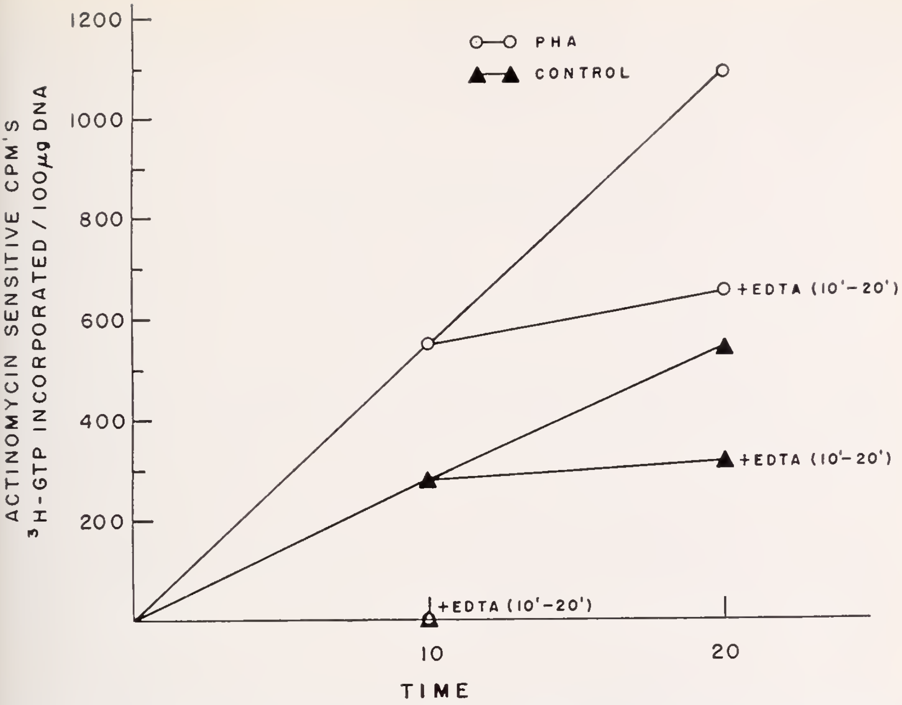

FIG. 5. Fate of H_3 labelled RNA produced by lymphocyte nuclei in the presence of exogenous RNA polymerase. Nuclei, from lymphocytes incubated with and without PHA, were isolated and incubated with RNA polymerase and H_3GTP as described in the text. EDTA (10 µmoles) was added at the indicated times.

showed the same threefold increase in the priming capacity of PHA-stimulated nuclei. Therefore, the increased ability of PHA nuclei to prime for RNA production did not appear to be secondary to an increased permeability of the nuclear membrane.

These differences might, however, be due to a differential destruction of the RNA produced *in vitro*. Addition of 10 µmoles of EDTA at the start of experiments abolished the cation-dependent production of RNA by RNA polymerase (Figure 5). Therefore, nuclei were incubated for 10 minutes to permit incorporation of H_3GTP into RNA; 10 µmoles of EDTA were then added and the reaction mixture incubated for a further 10 minutes to permit breakdown of newly synthesized RNA. At 10′ the control nuclei primed for incorporation of 281 CPM and after a further 10′ in the presence of EDTA, there were 314 CPM's remaining. The nuclei from PHA-stimulated cells showed a similar lack of degradation with 550 CPM's present after 10′ and 656 CPM's present after a further 10′ incubation in the presence of EDTA. Thus neither control nor PHA-stimulated nuclei showed any significant differential breakdown of newly made RNA under these conditions.

The apparent difference in priming capacity of nuclei might result from the presence either of inhibitors or activators of the priming reaction. Therefore, nuclei from control and stimulated cells were mixed after isolation and subsequently assayed. Whereas control nuclei primed for the incorporation of 372 CPM of H_3GTP and stimulated nuclei for 948 CMP, the mixture of nuclei incorporated an intermediate amount. Thus, no evidence for inhibitory or enhancing activity was obtained in either preparation.

Effect of Trypsin upon Template Capacity of Nuclei Isolated from Resting and Stimulated Lymphocytes

As one means of examining the DNA-protein relationships in stimulated *versus* non-stimulated cells, the effect was studied of trypsin upon the template activity of nuclei for bacterial RNA polymerase. Nuclei were incubated with trypsin at concentrations ranging from 1.25–25 μg/ml. The reaction was stopped by the addition of soybean trypsin inhibition at twice the concentration of the trypsin. Trypsin (TRL 100S 7JA) and crystalline Soybean Trypsin Inhibitor were obtained from Worthington Biochemical Co. In nuclei from PHA-treated cells the usual, approximately three to fourfold (3.56 ± 0.507) increases in template activity were noted. When such nuclei and those from cells not treated with PHA for 2 hours were subsequently exposed to trypsin (Table II), the template activity of nuclei from PHA-treated cells was not augmented by trypsin to the same relative degree as that of nuclei from control cells (for example: 1,370 to 25,500 for PHA, 370 to 12,200 for control). Consequently, the ratio of template capacity of nuclei from PHA-treated cells to that of nuclei from control cells (PHA/C ratio, Table II) decreased at all trypsin concentrations. Indeed the ratio decreased from 3.56 ± 0.507 (Table II) in the absence of trypsin to 1.7 ± 0.110 (mean value for all trypsin concentrations) in the presence of trypsin, a difference significant at $P < .005$.

Additionally, we have calculated the absolute increase in H_3GTP incorporation into RNA produced by trypsin in PHA-nuclei and control nuclei. Thus, trypsin treatment of nuclei isolated from PHA-stimulated lymphocytes induced greater *absolute* increments in H_3GTP incorporation into RNA (8,675 CPM at 1.25 μg trypsin/ml—24,130 CPM at 25 μg trypsin/ml) than did treatment of nuclei isolated from resting cells (4,950 CPM at 1.25 μg trypsin/ml—11,830 CPM at 25 μg trypsin/ml). This difference in *absolute* increments in H_3GTP incorporation induced by trypsin treatment was significant (paired t test) at $P < .025$. These experiments indicate that nuclei showed their greatest template activity when prepared from PHA-stimulated cells and when subsequently treated with trypsin.

Events following PHA Stimulation

These data indicate that stimulation of lymphocytes by phytohemagglutinin increases the ability of their isolated nuclei to act as templates for exogenous (bacterial) RNA polymerase. Several alternate explanations for this apparent increase in the priming capacity of the nuclei must, however, be considered. The observed rise in incorporation of H_3GTP into RNA could result from changes in nuclear architecture or contents, such as: (1) an increased permeability of the nuclear membrane to nucleotides or enzymes; (2) the action of

TABLE II

*Effect of Trypsin upon the Template Capacity of Nuclei Isolated from Phytohemagglutinin and Non-Stimulated Human Lymphocytes**

(Trypsin) μg/ml	No. of Expts.	PHA	Control	P/C Ratio
0	9	†1,370 ± 225	†370 ± 24	‡3.56 ± 0.507
1.25	2	10,050 ± 550	5,320 ± 343	1.89 ± 0.071
10.70	3	14,100 ± 251	8,880 ± 156	1.60 ± 0.102
12.50	2	13,250 ± 150	10,450 ± 1,909	1.29 ± 0.150
25.00	2	25,500 ± 4,500	12,200 ± 1,300	2.08 ± 0.145
		Average with trypsin		‡1.70 ± 0.110

* Cells were incubated with and without PHA for 2 hours and nuclei isolated. Nuclei were incubated for 20′ at 37°C with trypsin (1.25–25 μg/ml) and the reaction stopped by addition of soybean trypsin inhibitor (2.5–50 μg/ml). The capacity of treated nuclei to prime for incorporation of H_3GTP into RNA in the presence of exogenous RNA polymerase was determined (the conditions of assay were as described in the text). Values represent Actinomycin D-sensitive CPM H_3GTP incorporated/100 μg DNA ± SEM. The amount of DNA per assay was varied between 10–20 μg for trypsin treated nuclei and between 10–40 for non-trypsin treated nuclei. PHA/C ratio = incorporation of H_3GTP into RNA by nuclei from PHA-treated cells/incorporation of H_3GTP into RNA by nuclei from untreated cells.

† = $P < .005$ (paired t test) PHA *versus* control.

‡ = $P < .005$ (t test) P/C ratio without trypsin *versus* trypsin average.

soluble activators or inhibitors upon the exogenous enzyme; or (3) differential rates of degradation of products (RNA). These three possibilities have been examined experimentally. It was found that six cycles of freezing and thawing or treatment with Triton X-100 did not abolish the observed increase in apparent RNA synthesis by PHA-treated nuclei. Although the precise state of the nuclear membrane in these preparations was not evaluated by ultrastructural means, both of these procedures suffice to disrupt biomembranes in general, and indeed Triton X-100 has been shown to disrupt the outer nuclear membrane (36). Since these treatments did not alter observations made with intact nuclei, increased permeability of the nuclear membrane cannot be held responsible for the increased rate of RNA synthesis observed. It is furthermore possible that there exist, in the intact cell or nucleus, activators or inhibitors of RNA polymerase, or of the transcriptive capacity of DNA itself. However, since mixing of nuclei from PHA-stimulated and from non-stimulated lymphocytes resulted in a rate of incorporation of H_3GTP into RNA which was intermediate in level between that observed for either nuclear suspension alone, it would appear unlikely that activators or inhibitors played a role in the increased rate of incorporation. Moreover, no differential degradation of H_3 labelled RNA was observed; such data would seem to exclude the possibility that this early effect of PHA is mediated by nucleases. Finally, the presence of all four unlabelled nucleotides in abundance, in addition to H_3GTP, would make unlikely the possibility that the observed changes were due to alterations in the availability of labelled precursor (pool-changes).

The observed changes could also result from alterations in the relationship of DNA to either endogenous or exogenous enzyme. For example, the observed increased incorporation of H_3GTP into RNA could be due to: (1) an increase in the activity of endogenous RNA polymerase; (2) an increased rate of synthesis at sites on DNA previously available to the enzyme; or (3) the appearance of additional sites newly available for transcription of RNA. Although there may be an increase in endogenous RNA polymerase, the conditions of the assay were such that the levels of endogenous polymerase were insufficient to cause detectable incorporation of nucleotides into RNA either by control or stimulated nuclei. Additionally, amounts of added enzyme (exogenous polymerase) were so high as to render insignificant any increase in the much smaller amount of endogenous enzyme. Therefore, it would seem more likely that the increased capacity for RNA synthesis demonstrated here is secondary to alterations other than an increase in endogenous RNA polymerase. We have no direct evidence that increased rates of RNA synthesis in the presence of added polymerase are not simply due to increased rates at previously available sites on the DNA. However, evidence primarily derived from chromatin, recently reviewed by Bonner et al (25), suggests that nucleotide incorporation into RNA under these conditions does reflect the proportion of DNA available for transcription in the whole cell. The actual experimental data is neither as extensive nor as complete for nuclei as for chromatin, and is primarily correlative in nature. For example, Pogo, Allfrey, and Mirsky (37) have demonstrated an increased template activity of nuclei from regenerating, as compared to normal liver, a system in which Church and McCarthy (38), have demonstrated the production of new species of RNA. Hybridization studies of the RNA produced by isolated nuclei (although without added RNA polymerase) would suggest that this RNA produced *in vitro* is specific for the DNA used (39).

Therefore, the data presented above would suggest that, following stimulation of peripheral blood lymphocytes by PHA, more of the lymphocyte DNA is available for transcription into at least partly new RNA. Obviously, it remains to be demonstrated that some of this RNA does indeed represent new messenger RNA.

Several other very early changes following PHA stimulation have been described, and these resemble changes found in regenerating liver. These include an increased acetylation of histones (23) and an increased turnover of phosphoproteins (24). Such changes have been interpreted to represent early alterations in DNA-protein relationships which permit transcription of a greater portion of the genome. Although these modifications of nuclear proteins may well be the means whereby template activity is increased, and increased synthesis of RNA results, it is uncertain whether they are the primary site of action of PHA, or are a secondary link in the chain of events launched by the mitogen. We (28) have previously reported increases in the uptake (presumably by endocytosis) of molecules such as neutral red from the ambient medium. Indeed, perhaps as a reflection of enchanced endocytosis, increased synthesis of membrane-associated lipids has been found (40, 41). Consequent to these, we have shown a redistribution of hydrolytic enzymes from a latent, granular form, to the unsedimentable fractions of the cell. It is therefore possible that as a result of enzyme redistribution, a previously latent enzyme, resembling trypsin in gross effects, may have gained access to the nucleus. It would appear that exchange of components between nucleus and cytoplasm is possible not only during dissolution of the nuclear envelope, but also during stages at which a complete nuclear membrane exists (42).

After treatment of nuclei with trypsin, the ratio of the template activity of stimulated nuclei to that of non-stimulated nuclei was decreased. This decrement was due to *relatively* greater increases produced by trypsin in the template activities of nuclei from unstimulated cells than of stimulated cells. One possible explanation for this finding is that trypsin-like activity induced by PHA had already provoked increases in template activity before isolation of the nuclei. Finally, the *absolute* increases in template activity produced by trypsin were greater in stimulated than in unstimulated nuclei. The latter observation suggests that early changes induced by PHA resulted in the exposure of additional nuclear sites at which the action of trypsin resulted in increased RNA synthesis.

Summary

Nuclei isolated from human peripheral blood lymphocytes stimulated by phytohemagglutinin exceed nuclei from unstimulated cells in their capacity to prime for nucleotide incorporation into RNA in the presence of exogenous (bacterial) RNA polymerase. Trypsin augmented the template capacity of nuclei from rabbit liver, and increased the template activity of nuclei from unstimulated human lymphocytes to a relatively greater degree than of those from stimulated lymphocytes. The data suggest that transformation of cells by phytohemagglutinin is accompanied by increments in the number of DNA sites available for transcription by RNA polymerase, and that such increments can result from exposure of nuclei to hydrolytic enzymes.

Acknowledgments

We would like to thank Kathrin Krakauer and Judith Grossmann for their excellent technical assistance. Dr. Daniel Weissberg helped with the early experiments in this study.

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Hydrolysis of Purine and Pyrimidine Ribosides and 2-Deoxyribosides with Liquid Hydrogen Fluoride*

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Mineral acid hydrolysis of RNA is usually carried out at 100°C for 1 hour, whereby only the free bases of the purine components are produced, while the pyrimidine constituents remain in the form of their nucleotides in 95 percent yield (1). In order to liberate both purine and pyrimidine bases, much more drastic conditions are required (for example, concentrated formic acid at 175°C) (2). However, a considerable deamination of cytidine to uracil occurs during this procedure (3). Hydrolysis with 12 N perchloric acid (4) causes less deamination, but recovery of pyrimidine bases was found to be incomplete (5).

For complete hydrolysis of deoxyribonucleic acid, both 12 N perchloric acid at 100°C and 88% formic acid at 175°C are recommended (6). Application of the latter method, however, was reported to cause deamination of cytidine. It was of interest to investigate an acid hydrolysis method which could be carried out at room temperature or below. Hydrogen fluoride seemed to be a promising reagent. It has been used at 18°C for deacylation of esters of polyhydric alcohols (7). In this report, the applicability of hydrogen fluoride for the hydrolysis of purine and pyrimidine ribonucleosides and deoxyribonuceleosides is examined.

Experimental

MATERIALS

Purine and pyrimidine D-ribosides and 2-deoxyribosides, as well as their corresponding bases, were obtained in highest purity and in chromatographic homogeneous form (Calbiochem, Los Angeles, California). Solvents were of reagent grade and were used without further purification. Liquid anhydrous hydrogen fluoride was purchased from Matheson Co., Inc., East Rutherford, N.J.

PAPER CHROMATOGRAPHY

Ascending and descending techniques were carried out on Whatman No. 1 chromatographic papers at ambient temperatures. Starting positions for the ascending technique were at 5 cm from the bottom edge, and for the descending ones, 10 cm from the top edge of the paper sheets.

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†Deceased, 1969.

SOLVENT SYSTEMS

(1) Acetonitrile—water—dimethylsulfoxide = (12:4:1); (2) acetonitrile—dilute ammonia, pH 10 = (7:3); (3) dilute ammonia (1 N) added to water until pH 10; (4) n-butanol saturated with water; (5) n-butanol—glacial acetic acid—water = (50:25:25); (6) n-butanol—formic acid (88%)—concentrated ammonia = (77:10:13); (7) tertiary butanol—methyl ethyl ketone—formic acid (88%)—water = (40:30:15:15); (8) tertiary butanol—methyl ethyl ketone—concentrated ammonia—water = (4:3:1:2); (9) upper phase of the mixture: secondary butanol—tertiary butanol—water = (43:8.6:48.4); (10) n-dimethyl formamide—n-butanol—water = (1:1:1); (11) ethyl acetate—dimethyl formamide—water = (30:6:1); (12) ethyl acetate—formic acid (88%)—water = (7:2:1); (13) ethanol—water—dimethyl sulfoxide = (50:50:1); (14) isopropanol—water = (1:1); (15) isopropanol—concentrated ammonia—0.1 M boric acid = (35:5:10); (16) isopropanol—water—dimethyl sulfoxide = (12:4:1); (17) isopropanol—water = 7:3; (18) isopropanol—n-butanol—water = (14:2:1); (19) 4% aqueous sodium acetate.

THIN LAYER CHROMATOGRAPHY

Ascending thin layer chromatography (TLC) was performed on glass plates coated in 250 μ thickness with cellulose MN 300, crystalline cellulose MN 400, and polyphosphate cellulose 300 (Brinkmann Instruments Inc., Westbury, N.Y.) using Desaga spreader. Starting spots were applied at a distance of 3 cm from the bottom of the layers. The development was carried out at ambient temperatures at 90° angle from the spreading direction.

At least three solvent systems were applied to each hydrolyzate for simultaneous chromatography with the corresponding pure reference compounds. The spots were located over short-wave ultraviolet light (255 mμ). In addition to comparison of the R_f values, the ultraviolet spectrum of the spot extracts was used for identification. The position of D-ribose was visualized by staining with ammoniacal silver nitrate or with the combined aniline phosphate-diphenylamine reagent.

SPECTROPHOTOMETRY

Ultraviolet absorption spectra were obtained with the Cary Model II Recording Spectrophotometer at several pH values of the solution. The maxima, minima, and the characteristic absorbance ratios at 250, 260, 270, and 280 mμ were compared with the data obtained with pure compounds. When the hydrolysis product contained admixtures of unreacted starting material, assay of binary mixtures was applied to estimate the content of each component.

HYDROLYSIS PROCEDURE

Three experimental conditions were applied. In the first, strictly anhydrous conditions were maintained whereby atmospheric moisture was excluded by a current of dry air during addition of the liquid HF, and the nucleosides were carefully dried to constant weight over P_2O_5 *in vacuo*. In the second series, no special precautions were taken to insure anhydrous conditions, and in some experiments the substrates (for example, deoxyguanosine) were used in their hydrated form. In the third group, the substances to be hydrolyzed were moistened to a content of 10% water with respect to the volume of HF to be used.

The compounds were weighed into the bottom of plastic test tubes in such a manner that spreading of material on the walls was excluded, avoiding escape from reaction with the liquid HF. All operations with liquid hydrogen fluoride were performed under an efficiently ventilated hood, observing all necessary safety precautions (Chemical Safety Data Sheet SD-25, Manuf. Chem. Assoc. Inc., Washington, D.C.). Liquid anhydrous hydrogen fluoride was poured from previously cooled, inverted compressed gas cylinders of lecture bottle size into a polyethylene beaker. Portions of the liquid hydrogen fluoride, roughly measured with a polyethylene dipper, were added at 0°C to the material to be hydrolyzed.

The hydrolysis procedure may be illustrated by the following example: To the plastic test tube containing 20 mg of adenosine and cooled in an ice water bath were added 2 ml of liquid hydrogen fluoride. The mixture, frequently

TABLE I

Hydrolysis of 2-Deoxyribosides

	Experimental condition[1]*	Product	Yield %	Chromatography				
				Method[2]*	Solvent	R_f Values		
						Product	Base[3]*	2-Deoxyriboside[3]*
2-Deoxyadenosine monohydrate	2	Adenine	98	PA	1	0.48	0.48	0.59
	3	Adenine	75	PD	10	0.65	0.65	0.73
2-Deoxyinosine	2	Hypoxanthine	86	PA	3	0.75	0.74	0.85
				PA	8	0.34	0.37	0.50
				PD	H_2O	0.72	0.72	0.78
	3	Hypoxanthine	85	Same solvents		Comparable values		
Thymidine	2	Thymine	76	PD	13	0.78	0.76	0.85
				PD	11	0.64	0.63	0.74
	3	Thymine	94	Same solvents		Comparable values		
	2	Thymine		TLC PP	13	0.82	0.86	0.92
2-Deoxycytidine hydrochloride	2	Cytosine	75	PD	H_2O	0.72	0.72	0.82
			85	PD	H_2O	0.70		
				TLC PP	2	0.48	0.47	0.60
2-Deoxyguanosine monohydrate	2	Guanine	83	PD	H_2O	0.41	0.40	0.67
				PA	2	0.21	0.21	0.40
	3	Guanine	74	Same solvents		Comparable values		

* 1: see text for hydrolysis procedure; 2: P = paper chromatography, A = ascending, B = descending, TLC PP = thin layer chromatography on cellulose polyphosphate; 3: R_f values obtained from pure compound simultaneously with the products.

stirred, remained in the ice water bath for 5 hours. The bulk of the liquid was then allowed to evaporate under the hood in a current of dry air. After 32 hours the tube was placed in a desiccator containing sodium hydroxide pellets, and the residual liquid was removed *in vacuo*. The dry residue was dissolved in water, and the solution examined by ultraviolet absorption spectrophotometry, paper, and TL chromatography. The hydrolysis products from guanosine and deoxyguanosine were dissolved in 0.1 N sodium hydroxide, and those from xanthosine in 0.1 M lithium carbonate.

Results and Discussion

When moisture was completely excluded (first procedure) during hydrolysis with anhydrous liquid hydrogen fluoride, inhomogeneous products were obtained. Paper chromatography revealed multiple spots that absorbed and stained with ammoniacal silver nitrate. This indicates the presence of decomposition or transformation products of the purine, pyrimidine, and carbohydrate moieties. These products were not further investigated.

Evidently, water is necessary for uniform hydrolysis. With access of atmospheric moisture, and also in the presence of water of crystallization (as in the case of deoxyguanosine monohydrate), the products were satisfactory. Homogeneous hydrolyzates, with respect to the liberated bases, were obtained from all deoxyribosides so far investigated and from ribosides, with the exception of cytidine. The latter did not react completely with hydrogen fluoride, as shown by the presence of varying amounts of unhydrolyzed material in several experiments. The cause of this exemption is obscure.

DEOXYRIBOSIDES

Hydrogen fluoride (under experimental conditions (2, 3)) liberates the bases from both purine and pyrimidine deoxyribosides between 0°C and room temperature, in yields ranging from 75 to 98 percent of the theoretical (Table I). The deoxyribose moiety is destroyed, forming an insoluble black residue. This charcoal-like residue apparently irreversibly absorbs some of the bases, which may explain their lower and irregular yields. "Charcoal" formation by hydrolysis of deoxypentose nucleic acid, with 70% perchloric acid at 100°C for 1 hour, was also observed by Levenbrook, as quoted by Wyatt (5).

Table I presents examples of the chromatographic data for the hydrolyzates of deoxyribosides of adenine, cytosine, guanine, hypoxanthine, and thymine. The solvent systems listed were found to be especially suitable for distinguishing the bases from their deoxyribosides. The R_f value of each product coincided with that of the corresponding base used as reference. Each chromatographic identification was further confirmed by the agreement of the ultraviolet absorbances and their characteristic ratios.

RIBOSIDES

The reaction of hydrogen fluoride on ribosides (Table II) produced free bases and the equivalent amount of intact D-ribose. The latter could be dem-

TABLE II

Hydrolysis of Ribosides

	Experimental Condition[1*]	Product	Yield %		Chromatography						
							Rf Values				
			UV Assay	Orcinol Assay	Method[2*]	Solvent System	Product	Reference		Sugar	
								Base[3*]	Riboside[3*]	Product	D-Ribose[3*]
Adenosine	2	Adenine	89	92	PA	5	0.62	0.64	0.53	0.43	0.42
	2		113	100	PA	8	0.46	0.47	0.32		
					TLC PP	H_2O	0.28	0.31	0.50		
					TLC PP	14	0.49	0.49	0.62		
	3		103	96	PA	9	0.76	0.78	0.66	0.56	0.51
Guanosine	2	Guanine	106		PA	15	0.51	0.51	0.67		
	2				PD	H_2O	0.37	0.37	0.59		
					PD	11	0.25	0.23	0.35		
			98		PA	15	0.52	0.52	0.66		
					PD	H_2O	0.36	0.38	0.61		
Inosine	2	Hypoxanthine	95		PA	4	0.25	0.27	0.10	0.18	0.18
	2		106	105	PA	6	0.32	0.29	0.16	0.20	0.18
	3		99		PA	18	0.40	0.44	0.30	0.45	0.46
Xanthosine	2	Xanthine	97		PD	17	0.67	0.67	0.84		
					PA	3	0.72	0.74	0.81		
					PD	19	0.44	0.44	0.62		
Cytidine hemisulfate	2	Mixture									
		I Cytosine	32	30	PA	12	I 0.50	0.49	0.29	0.40	0.40
		II Cytidine					II 0.25				
					PA	H_2O	I 0.62	0.64	0.72		
							II 0.70				
					PD	5	I 0.48	0.50	0.39	0.43	0.41
							II 0.37				
	3	Unreacted cytidine			PD	5	0.40		0.39		
						12	0.29		0.29		
Uridine	2	Uracil	97		PD	5	0.50	0.49	0.35	0.40	0.40
					PA	12	0.62	0.64	0.40	0.40	0.37
					PA	7	0.58	0.58	0.47	0.43	0.43
	3	Mixture									
		I Uracil	48		PA	8	I 0.59	0.56	0.47	0.66	0.63
		II Uridine					II 0.49				

* 1: see text for hydrolysis procedure; 2: P = paper chromatography, A = ascending, B = descending, TLC PP = thin layer chromatography on cellulose polyphosphate; 3: Rf values obtained from pure compound simultaneously with the products.

onstrated simultaneously with their corresponding bases on paper chromatograms and determined quantitatively in the hydrolyzates by the orcinol reaction (8, 9), confirming the yields of the bases as estimated from their ultraviolet absorbances.

Liberation of free bases in yields ranging from 90–100 percent (Table II) was achieved by both procedures (100 and 90% hydrogen fluoride) from adenosine, guanosine, inosine, and xanthosine. Uridine afforded practically homogeneous uracil only with procedure 2, while 90% hydrogen fluoride gave mixtures of uracil with unreacted uridine.

Difficulties were encountered with cytidine. In most experiments, procedure 2 produced mixtures of cytosine (30%) and cytidine. Only in 1 experi-

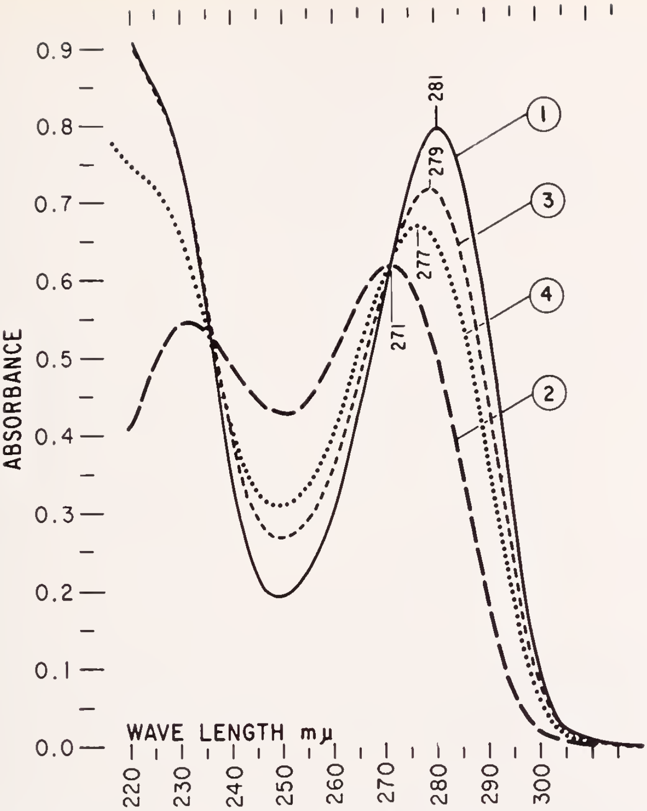

Fig. 1. Ultraviolet spectra of solutions in 0.16 M NaOH: ① = 10 × 10^{-5} M cytosine; ② = 7 × 10^{-5} M cytidine; ③ = mixture: 7.5 × 10^{-5} with respect to cytosine, and 0.175 M with respect to cytidine; and ④ = mixture: 5.0 × 10^{-5} with respect to cytosine, and 3.5 × 10^{-5} M with respect to cytidine.

ment, which could not be duplicated, was a yield of 72% cytosine obtained. Attempts to increase the yield by continuous stirring or by catalysis of the reaction with metal ions (for example, ferric, cupric, zinc) were not successful. Repeated application of anhydrous hydrogen fluoride to the reaction product increased the yield of cytosine to a total of 82 percent. By procedure 3 only 12% of cytidine was hydrolyzed.

The ultraviolet spectra for cytosine and cytidine at pH 12–13 are so different (Fig. 1, curves ① and ②) that it should be possible to estimate the concentration of each of these compounds in their mixtures. The ultraviolet absorbancies at two wave lengths from a given mixture, as well as the known molar absorptivity of each of the pure components at the same pH, supply sufficient data for solving two simultaneous equations based on Beer's Law. This method has been previously applied to binary mixtures of purine and pyrimidine bases (5), but not to mixtures of nucleosides with their corresponding bases. It was therefore tested on synthetic mixtures containing varying concentrations of cytosine and cytidine in 0.17 N NaOH. The absorbancies from their ultraviolet spectra (Fig. 1, curves ③ and ④) at 260 and 280 mμ gave very good results. When applied to one of the cytidine hydrolyzates obtained with anhydrous hydrogen fluoride, a value of 31.6% cytosine calculated from the absorbance values agreed very well with 30.0% D-ribose given by the orcinol colorimetric method. The same method was used for calculation of the concentrations of uracil and uridine mixtures obtained from the treatment of uridine with 90% hydrogen fluoride (Table II).

Summary

In order to develop methods for hydrolysis of nucleic acids at room temperature or below, the reaction of liquid hydrogen fluoride at 0°C with six ribo- and five 2-deoxyribonucleosides was investigated. Under specified conditions, 90 to 100% of adenosine, guanosine, inosine, xanthosine, and uridine were hydrolyzed to free bases and D-ribose. The hydrolysis of cytidine was incomplete. The yields of free bases from 2-deoxyribosides ranged from 90 to 100%. The deoxyribose moiety was destroyed. An application of a spectrophotometric assay method to binary mixtures of cytidine and cytosine, as well as of uridine and uracil, is described.

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Physics and Physiology of Transferrin

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Although the essential role of heavy iron in cellular metabolism has been recognized for well over 100 years, only recently has attention been paid to the transport mechanisms involved in the delivery of this essential metal from its site of absorption in the gut to its site of utilization in the tissues. The problem is that at the homeostatically fixed pH, ionic composition, and oxygen tension of blood, ionic iron cannot survive as such, but will autoxidize to the ferric state and "rust" out as insoluble ferric hydroxide. When tightly bound to a carrier, however, it can be maintained in solution in blood until it is utilized for the biosynthesis of the specific proteins (particularly hemoglobin) which require iron in their prosthetic groups. Furthermore, when complexed to specific metal-binding agents, iron is able to resist being bound to the nonspecific ion-exchanging groups which are so prevalent on the surface of cell membranes.

Low molecular weight chelating agents might well serve the transport function for ion in blood were it not for one further major advantage afforded by a specific transporting protein. This advantage is particularly well illustrated in the case of transferrin, which is capable not only of specifically binding iron, but of selectively delivering it only to those cells which require it for the synthesis of heme and other iron bearing proteins (1). Thus, transferrin meets the requirements for an ideal physiological iron carrier (Table I) for the organism with a circulatory system, and since it can be isolated and crystallized in pure form (2), it meets the requirements of the biochemist as well.

The interesting questions that may be asked of a metal-carrying protein are firstly, how does it specifically bind metal ions and, secondly, how are these metal ions released to the tissues? Ideally, of course, the detailed structural information necessary to answer these questions will be provided by x-ray crystallography. In the case of transferrin, however, with its high molecular weight, x-ray crystallography at the resolution necessary to obtain fine structural details is an extremely formidable undertaking with presently available methodology. Fortunately, other methods borrowed from the physical sciences can be brought to bear on these questions, and although final answers are not available from their utilization, biologically relevant information can be obtained with them. In particular, the techniques of magnetic resonance have been applied with some success to the study of the metal complexes of transferrin.

Magnetic Resonance Techniques in the Study of Biological Systems

A lucid and informative discussion of the value of electron paramagnetic resonance spectroscopy in the study of biological systems has been given by

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TABLE I

Requirements of an Ideal Physiological Iron Carrier

1. Relative specificity for iron, preferably Fe^{3+}, because of prevalence of other multivalent cations in blood.
2. Tight, but reversible, binding of iron.
3. Variable capacity to accommodate variations in demand for iron transport (for example, after hemorrhage).
4. Recognition function: to distinguish between cells with high requirements for iron (for example, reticulocytes) and cells with low requirements (for example, mature red cells).

Beinert and Palmer (3). For the purposes of this review, suffice it to say that electron EPR spectroscopy is in many ways analogous to the much more conventional and extensively utilized spectrophotometry. In the latter case, electronic transitions induced by the absorption of optical energy are observed and recorded as a function of the optical frequency (energy) or wavelength. In EPR spectroscopy, the intensity of the energy absorbed, in this case at microwave or radar frequencies, is displayed as a function of the magnetic field in which the observed species is placed. The energy scale is thus represented by a magnetic field scale. Because EPR spectroscopy observes transitions between energy levels induced by an externally applied magnetic field, its value lies in the study of compounds having permanent magnetic moments, that is, compounds with unpaired electrons. Two classes of such compounds are of interest in biology: (1) free radicals, in which one or more molecular orbits contain an unpaired electron; and (2) compounds bearing transition metal ions, in which unpaired electrons inhabit one or more atomic orbitals of the metal ion. The great value of EPR spectroscopy in the study of biological systems is that it can provide detailed information not only about the types of paramagnetic species present, but also about the neighborhood in which these paramagnetic species find themselves. The detailed presentation of the methods involved in deducing the kinds of information that are obtainable from electron paramagnetic resonance spectroscopy will not be given in this review, but the results that are often obtainable from these analyses will be illustrated in the discussions of the metal bearing protein of blood.

Transferrin

Transferrin, a protein of molecular weight 82,000 (Table II) punningly named for its function of transporting iron in blood, is characterized by two related properties of physiologic consequence: (1) It reversibly binds two iron atoms per molecule; and (2) It preferentially transfers its iron to immature red blood cells or reticulocytes which require it for the biosynthesis of hemoglobin. These properties afford a system, so far unique, for studying both the interaction of a protein with a metal, and a protein with a cell.

The transferrin molecule is saturated with iron when two atoms, each in the

TABLE II

Properties of Transferrin

1. Molecular weight 82,000; single polypeptide chain.
2. Two identical or nearly identical metal-binding sites per molecule.
3. Fe^{3+} bound more tightly than other metal ions.
4. Bicarbonate facultatively bound only when metal is bound.
5. Efficient iron donor for reticulocyte, but not for mature red cells.
6. Capacity to bind metals other than iron, including copper, manganese, chromium, and cobalt.

ferric state, are bound. For each iron bound, three protons are released from the protein molecule, and one bicarbonate ion is simultaneously bound (4). The reaction of binding then can be described by the following equations:

$$Fe^{3+} + H_6Tfn + HCO_3^- \rightleftharpoons (Fe^{3+}H_3Tfn \cdot HCO_3)^- + 3H^+ \tag{1}$$

$$Fe^{3+} + (Fe^{3+}H_3Tfn \cdot HCO_3)^- + HCO_3^- \rightleftharpoons [(Fe^{3+})_2Tfn\ (HCO_3)_2]^{2-} + 3H^+ \tag{2}$$

From these equations it is possible to deduce expressions for the two thermodynamic binding constants which describe the affinity of each binding site for ferric ion. Furthermore, it is evident from the fact that three protons are released from the protein and one bicarbonate is bound to it for each iron atom bound, that the net negative charge of the protein increases by one for each iron atom bound. These properties are utilized in determining the intrinsic affinities of the two binding sites for ferric ions.

Equilibrium dialysis studies by Malmström and his group (4) indicated that the affinities of the two metal-bindings of transferrin for ferric iron were approximately equal. If so, this leads to the prediction that three species of transferrin molecules should be identifiable at various saturations with iron. Using the technique of free electrophoresis, in an instrument modified so as to produce maximum resolution of species with nearly identical electrophoretic mobilities, it was possible to identify the three species predicted from the Malmström experiments (5). At zero or 100 percent saturation with iron, the protein migrates as a single homogeneous species in the electrophoretic apparatus. The iron saturated transferrin has a faster anodal mobility then does the apoprotein, because of the increase in net negative charge induced by iron binding. At intermediate degrees of saturation with iron, however, more than one species of protein molecule is identifiable in the electrophoretic pattern. At low degrees of saturation with iron there is a slow moving component, which has a mobility very close to that of apotransferrin, plus a faster moving component, which has a mobility intermediate between that of apotransferrin and iron saturated transferrin. At less than 50 percent saturation with iron the relative magnitude of this intermediate component increases with the degree of iron saturation, while the magnitude of the component with the mobility of apotransferrin correspondingly decreases. At more than 50 percent saturation, on the other hand, the magnitude of the intermediate component decreases with increasing iron saturation, while the magnitude of the com-

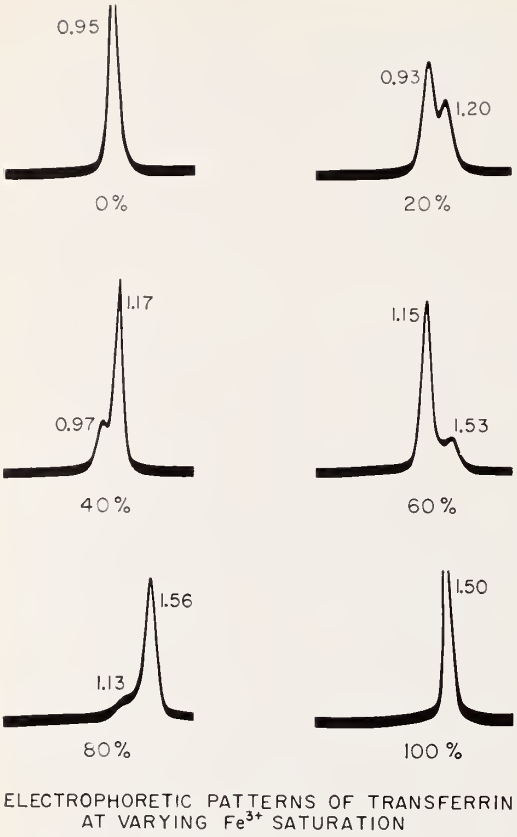

FIG. 1. Moving boundary electrophoresis diagrams of transferrin at varying degrees of saturation with Fe^{3+}. Numbers indicate mobilites of adjacent peaks, in units of -1×10^{-5} cm^2/volt/second; percentages indicate approximate degree of saturation with Fe^{3+} (reprinted by permission from Aisen, P., Leibman, A., and Reich, H. A.: Studies on the Binding of Iron to Transferrin and Conalbumin, J Biol Chem 241:1666, 1966).

ponent corresponding to the Fe-saturated protein correspondingly increases (Fig. 1). It seems clear, therefore, that this intermediate species corresponds to the transferrin molecule with only one iron atom bound. Furthermore, the appearance of the electrophoretic patterns at varying degrees of iron saturation confirms the finding from equilibrium dialysis studies that the binding sites have approximately equal affinities for iron atoms.

It seems reasonable to ask now whether the two sites are truly identical, since they have virtually identical binding affinities for iron. Because of the random nature of the binding imposed by the identical binding constants, it becomes impossible to sort out the contribution of each site in ordinary spectrophotometric titration experiments. To answer this question the technique of electron paramagnetic resonance (EPR) spectroscopy must be utilized, with its far greater sensitivity to structural details. In applying EPR spectroscopy to the study of transferrin, it will also become possible to answer other questions about the nature of metal-binding by transferrin, and perhaps even of the mechanism of metal release by transferrin under physiologic conditions.

The characteristic salmon-pink color of iron-transferrin complex is developed only in the presence of bicarbonate. For this reason it has long been assumed that bicarbonate ion was essential to the binding of iron by transferrin. We set out to determine whether this indeed is the case, taking advantage of the sometimes extraordinary sensitivity of EPR spectroscopy to changes in the environment of metal ions. Apparatus was constructed which permitted the addition of ferric ions to transferrin under CO_2-free, and therefore bicarbonate-free conditions (6). As expected, and as observed before, no color developed. In the EPR spectrometer, however, a characteristic spectrum of iron in an environment with particular symmetry (rhombic) was seen. The appearance of this spectrum provided strong evidence that iron was now in a specific metal-binding location, as iron nonspecifically bound to protein did not produce a signal with the characteristics and intensity of the observed signal. Furthermore, on standing under CO_2-free conditions, the intensity of the signal from this binary Fe^{3+}-transferrin gradually diminished and a precipitate of ferric hydroxide appeared. It seemed, therefore, that in the absence of bicarbonate, the iron-protein bond was sufficiently weakened so that hydrolysis of the ferric ion could occur. In the presence of bicarbonate, by contrast, the iron-protein bond is extraordinarily stable.

Since bicarbonate is not essential for the binding of iron by transferrin, but is only facultatively bound when present, is it possible to replace bicarbonate ion with other ions? This question was examined by offering iron to apotransferrin, not in its free form, but as its complex with a low molecular weight chelating agent. When oxalate or nitrilotriacetate were the chelating agents chosen, the characteristic red color expected from the transferrin-iron-bicarbonate complex developed almost immediately (6). The spectrophotometer, then, was unable to provide evidence of replacement of bicarbonate ion by other agents. When the complexes that resulted from the addition of the ferric chelates to transferrin were examined in the EPR spectrometer, spectra were obtained which were substantially different from those given by the usual iron-bicarbonate-transferrin spectrum or by the binary iron-transferrin complex in the bicarbonate free form (6). Furthermore, when bicarbonate ion was added to these new chelate complexes of transferrin, the spectrum more or less rapidly reverted to that given by native iron-transferrin-bicarbonate. Even simply allowing the preparation to stand in air so that CO_2 could be absorbed resulted in complete and quantitative conversion of the spectrum to the iron-transferrin-bicarbonate spectrum.

Another property of transferrin which provides a useful handle for investigating the structure of its metal-binding sites is the protein's ability to accommodate ions other than iron. Copper, cobalt, manganese, and chromium are all tightly bound to the protein in pairs, and for each of these metal ions bound a bicarbonate ion is concomitantly bound (7), providing excellent indication that the binding of these ions is to the specific metal-binding sites. Further confirmation of this is given by the finding that iron will displace each of these metal ions from its complex with transferrin. Study of the copper complex of transferrin by EPR spectroscopy is particularly revealing, since the

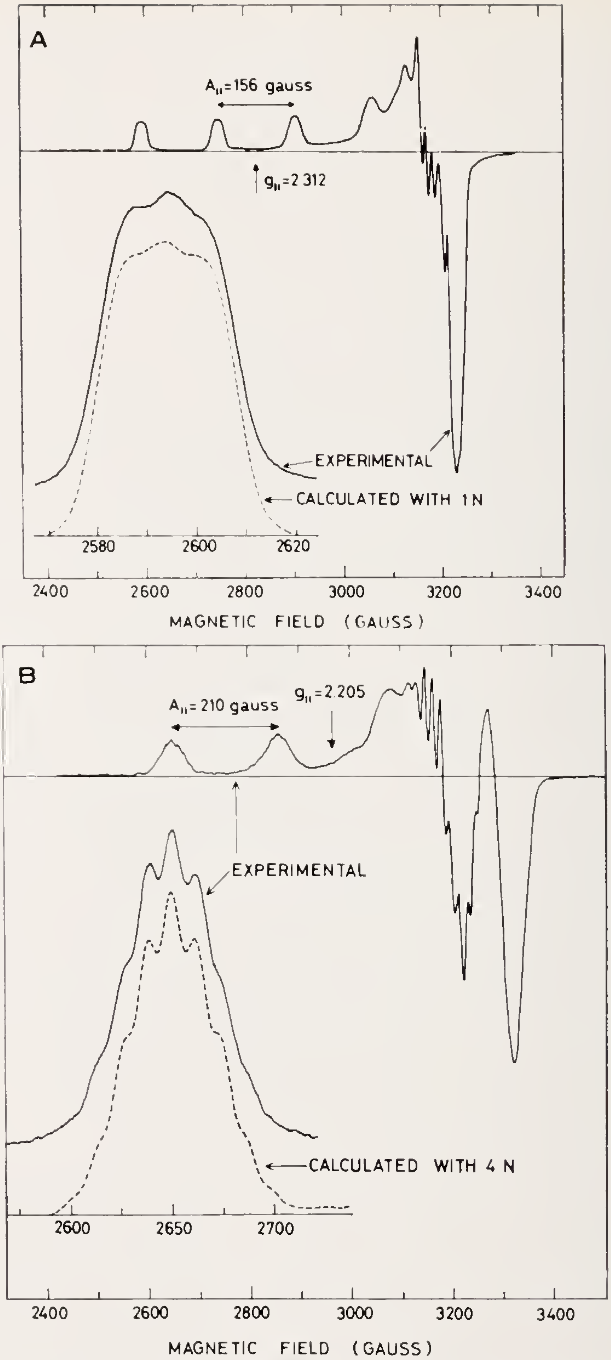

Fig. 2A. EPR spectrum of copper-transferrin-bicarbonate. The copper hyperfine line at lowest field is shown with 10 times increased gain in an expanded field scan; the dashed curve is a computer simulation assuming interaction of the copper electron with one nitro-copper ion is the simplest of all these transition metals ions (8). It contains only one unpaired electron (hole), in contrast to iron which contains five unpaired electrons. Accordingly, the spectrum of copper-transferrin is far more easily interpreted utilizing quantum mechanics than is the iron spectrum. The information that may be obtained by electron paramagnetic resonance spectroscopy is well illustrated in the study of copper-transferrin. The spectrum shows the characteristic four line structure that is the signature of cupric ion in the EPR spectrometer (3, 7). Closer inspection reveals, however, that these four lines have an unusual structure, best seen in the first or low field line. It is actually composed of three nearly superimposed, but nevertheless resolvable individual lines. The separation between these lines is about 10 gauss. Theoretical analysis indicates that such a structure would be produced by the interaction of the single unpaired copper electron with the nucleus of one nitrogen atom. This is corroborated by a computer simulation of the spectrum which assumes the interaction of the copper electron with a nitrogen nucleus according to the principles of quantum mechanics. Making this assumption, it is possible to simulate almost exactly the spectrum of the copper complex of transferrin (Fig. 2A). A study was also undertaken of the bicarbonate-free copper complex of transferrin in the EPR apparatus. Now the spectrum shows considerably more structure, and the low field hyperfine line is resolvable not into three components, but into nine components, again with a separation near 10 gauss (8). A computer simulation of the spectrum assuming interaction of the copper electron with four equivalent nitrogen nuclei almost exactly duplicates the experimental spectrum (Fig. 2B). It seems clear, therefore, that four nitrogen containing ligands are present at each metal-binding site, and the remarkable strength of binding of metal ion to transferrin is probably accounted for in large part by the well-known affinity of nitrogen ligands for transition metal ions. Why one nitrogen nucleus is seen in the bicarbonate complex while four such nuclei are seen in the bicarbonate-free complex remains unexplained. Evidently, however, the bicarbonate profoundly affects the structure of the metal-binding site, as well as the tightness of the metal-protein bond. It seems not unlikely that this unusual phenomenon must have physiologic significance.

Since chromium is another ion readily accessible to study and analysis by EPR spectroscopy, the specific chromium complex of transferrin was also prepared and its spectrum recorded (7). Theoretical analysis of this spectrum assuming only one kind of chromium ion to be present proved to be impossible. It was necessary to invoke the presence of two slightly but significantly dif-

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gen nucleus (reprinted by permission from Aasa, R., and Aisen, P.: Electron Paramagnetic Resonance Study of the Iron and Copper Complexes of Transferrin, J Biol Chem 243:2399, 1968).

FIG. 2B. EPR spectrum of copper-transferrin in absence of bicarbonate. The copper hyperfine line at lowest field is shown with 10 times increased gain in an expanded field scan; the dashed curve is a computer simulation assuming interaction of the copper electron with four nitrogen nuclei (reprinted by permission from Aasa, R., and Aisen, P.: Electron Paramagnetic Resonance Study of the Iron and Copper Complexes of Transferrin, J Biol Chem 243:2399, 1968).

ferent kinds of chromium ion; that is, chromium ions present in two slightly different configurations of the binding sites. Each chromium ion gives rise to its own spectrum slightly different from that produced by the other; the observed spectrum is a composite of that produced by each ion. If so, this means that the binding sites of transferrin are slightly different, at least when chromium ion is bound to the protein. That this is indeed the case is shown by displacement experiments in which the more tightly bound ferric ion is added to the chromium complex. The added ferric ion slowly displaces each chromium ion from its specific binding site. However, the ions are displaced sequentially rather than simultaneously so that the spectrum produced by one is obliterated before the spectrum produced by the other is. What finally results is the spectrum produced by the nonspecifically bound chromium ions. Thus, the chromium ion serves as a marker for displaying a slight but significant difference between the metal-binding sites with transferrin. The physiological counterpart of this experiment is seen in an elegant study of Fletcher and Huehns (9), in which a difference in the ability of each binding site of transferrin to donate its iron atom to the reticulocyte was demonstrated. This binding is particularly interesting and unexpected because the binding constants for the two sites are so nearly the same.

Electron paramagnetic resonance spectroscopy applied to the study of this biologically interesting macromolecule thus provides the first direct information about the chemical nature of the metal-binding site. Each site is shown to contain at least four nitrogen ligands, and each site differs from its counterpart in the protein molecule in a subtle, and as yet unexplained, way. Probably more detailed information will be provided only by x-ray crystallographic methods. Furthermore, how a protein composed of a single polypeptide chain comes to have two nearly identical and functionally specific metal-binding sites is a question of considerable genetic interest. What concerns us now is the mechanism by which transferrin interacts with the reticulocyte to deliver its iron to the hemoglobin synthesizing apparatus of the cell.

The Transferrin-Reticulocyte Interaction

Under physiological conditions of pH and bicarbonate ion activity, the apparent stability constant for the binding of iron to transferrin is approximately 10^{24}. From the magnitude of this affinity constant, it can be shown that on the average the time required for an iron atom to dissociate spontaneously from its binding site on the transferrin molecule would be something of the order of 10,000 years (10). It is abundantly clear, therefore, that the mechanism of iron release from transferrin cannot be a simple process of chemical diffusion. This is experimentally verified by labelling transferrin with radioactive iron and incubating it with unlabelled transferrin which has been stripped of its sialic acid residues. The asialotransferrin can then be separated from the native transferrin by ion-exchange column chromatography. When this is done, no exchange of iron atoms between the different kinds of transferrin molecules is observed, even when the incubation period is extended

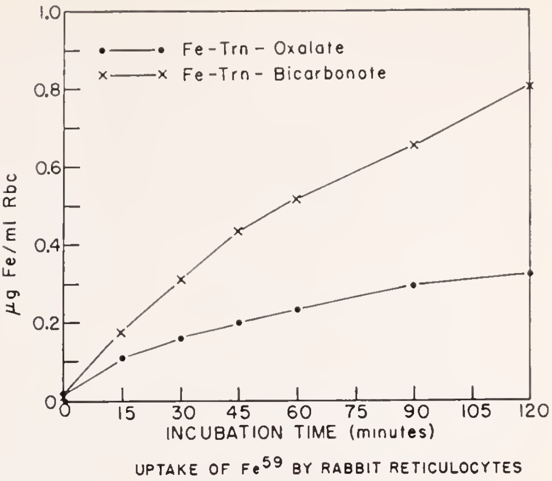

FIG. 3. Uptake of iron by rabbit reticulocytes from Fe-transferrin-bicarbonate and Fe-transferrin-oxalate.

over a period of several days as would be expected if spontaneous dissociation of iron did occur (10).

Saltman (11) has called attention to the probable role of small molecular weight chelating agents in the physiologic transport of iron. The possibility that citrate ion, which is ubiquitously distributed in physiologic fluids, could serve as a mediating agent for the exchange of Fe^{3+} between transferrin molecules was accordingly investigated. Physiologic concentration of citrate was shown to be capable of rapidly promoting exchange of Fe^{3+} among transferrin molecules (9). Could this then be the mechanism by which iron also exchanges between transferrin and the reticulocyte? Experiments by Jandl and his collaborators (1) seem to rule out this possibility, since the presence of excess chelating agents in no way perturbs the transferrin-reticulocyte interaction. Some other mechanism must be operative.

The EPR studies cited above indicated that bicarbonate was facultatively bound to transferrin whenever iron was bound, and that the binding of bicarbonate was essential for the tight binding of iron by the protein. Perhaps, then, the bicarbonate ion is somehow involved in the uptake of iron from transferrin. If the reticulocyte were capable of removing bicarbonate from the ternary complex of iron, transferrin, and bicarbonate, the iron-protein bond would be tremendously weakened, and the problem of removing iron from transferrin would cease to exist. This hypothesis was tested by comparing the efficiency of iron donation from transferrin molecules with bicarbonate, and transferrin molecules in which the bicarbonate had been replaced by oxalate. It was found that the natural iron-transferrin-bicarbonate complex was from 2 to 3 times better an iron donor then was the artificial iron-transferrin-oxalate complex (Fig. 3). This was so when free bicarbonate and no free oxalate was present. Thus, bicarbonate bound to transferrin seems somehow to be involved in the transferrin-reticulocyte site interaction. Whether the participation of bicarbonate involves the effect it has on the strength of the iron-protein bond, or whether it somehow facilitates the "recognition" function of transferrin is a subject for further experimentation.

The application of physical methods to the study of biological problems has made it possible to pose and sometimes even to answer questions that were previously unreachable. Admittedly, the speculations often outstrip the facts, and questions certainly exceed the answers. But this is not an unusual circumstance, and perhaps not even entirely undesirable.

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Stereospecific Drug Action and Receptor Theory

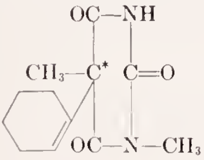
ENOCH GORDIS, M.D.

Optical antipodes of an asymmetric molecule can be distinguished in a chemical environment only if the environment itself has asymmetric constituents. Asymmetric metabolites such as amino acids function in the organism in only one enantiomeric form. Physiological discrimination between optical antipodes is due to enzymes. Enzymes are themselves asymmetrical, both because of their spatial configuration and because of the asymmetry of their component amino acids.

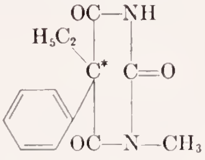
Many drugs which exist in two or more optically active forms act exclusively or preponderantly in one configuration rather than another. Among these drugs are alkaloids, narcotics, certain barbiturates, and drugs affecting autonomic and neuromuscular function. The asymmetric body constituents which discriminate among the enantiomeric forms of drugs are not known. They are usually assumed to be members of the class of "receptor" substances in tissues. According to receptor theory (1–3), drug-receptor interaction is necessary for drug action. To be effective, drug and receptor must be structurally suited to each other. Asymmetric loci on receptors distinguish between active and inactive forms of optically active drugs.

In current use, the word "receptor" represents a macromolecule, probably protein or nucleic acid (3). Most pharmacologists do not claim that receptors are enzymes, although some may be. Many receptors may display in their interaction with drugs certain enzyme-like features, such as competitive and non-competitive inhibition. In most cases it is not clear how the contact between drug and receptor leads to drug action. In a few instances, the chemistry of the drug-receptor reaction is known, such as the alkylation of nucleic acids by alkylating agents. In general, however, receptors have not been identified, and their properties are inferred from dose-response studies of isolated tissues.

The theory of macromolecular receptors has been found compatible with the action of many drugs and their analogues. Difficulties do arise, however, and the following example of stereospecificity is provocative:



(I)



(II)

*asymmetric carbon

Hexobarbital (I) and methylphenobarbital (II), each with one asymmetric

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TABLE I

Pharmacological Stereoselectivity of Barbiturate Enantiomers
(Büch et al, 1968)

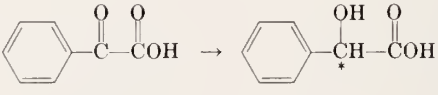
	Hexobarbital	Methylphenobarbital
Penetration into brain	Dextrorotatory	Dextrorotatory
Acceleration of disappearance from blood by phenobarbital pretreatment	Dextrorotatory	Dextrorotatory
Hypnotic effect	Dextrorotatory	Levorotatory

carbon, were resolved by Knabe (4, 5). Both pairs of antipodes were tested in rats by Büch and his colleagues (6) for penetration into the brain, acceleration of disappearance from blood by phenobarbital pretreatment, and hypnotic effect. The results are summarized in Table I.

The configuration of hexobarbital necessary for hypnotic effect is defined by that configuration necessary for maximum brain penetration and phenobarbital-induced acceleration of clearance from blood. It is difficult to see why this is not true for methylphenobarbital also if the receptors involved are the same. Of course, additional receptors can be postulated.

Reversal of antipodal specificity in a series of analogues is difficult to explain, but does not necessarily invalidate the idea of a macromolecular receptor. A similar difficulty arises with chymotrypsin (7). Chymotrypsin hydrolyzes most N-acyl alanine ester derivatives faster as their L-enantiomers, but the N-picolinyl derivative is hydrolyzed faster as the D-enantiomer (8). No entirely satisfactory explanation is yet available. Whatever accounts for the reversal in the case of chymotrypsin may also account for the reversals of drug stereospecificity. However, chymotrypsin is available and the receptors are hypothetical. It is worthwhile, therefore, to consider an alternative view of drug-receptor interaction.

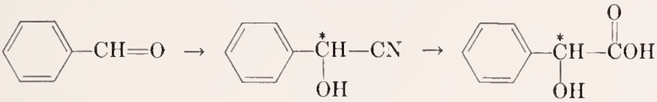
Many chemical reactions are known in which symmetric small molecules are transformed into a racemic mixture of optically active products (9). Some of these reactions yield an optically active mixture if conducted in an environment of optically active small molecules. Two well-known types of stereoselective reactions involving small molecules are partial asymmetric synthesis and asymmetric catalysis (9). An example of partial asymmetric synthesis is the reduction of phenylglyoxylic acid (III) to mandelic acid (IV):



*asymmetric carbon

If the reduction is conducted directly, racemic mandelic acid is produced. If, however, the phenylglyoxylic acid is first esterified with (−)-menthol, and the esters are then reduced and hydrolyzed, (−)-mandelic acid is produced in excess. The reason is that the reductions of the two diastereoisomers, (−)-menthyl(−)-phenylglyoxylate and (−)-menthyl(+)-phenylglyoxylate, proceed at different rates.

An example of asymmetric catalysis is the conversion of benzaldehyde (V) to mandelic acid (VII) via mandelonitrile (VI):



(V) (VI) (VII)

*asymmetric carbon

Racemic mandelic acid is formed, optically inactive. If the reaction is conducted in the presence of (−)-quinine, the product is levorotatory. If the reaction is conducted in the presence of (+)-quinidine, the product is dextrorotatory.

Asymmetric catalysis can be provided by an optically active solvent. For example, racemic s-butyl chloride was converted to optically active α-methylbutyric acid (via butylmagnesium chloride) when the reaction was conducted in (+)-2,3-dimethoxybutane (10). The solvent formed part of the optically active Grignard complex.

An alternative hypothesis to explain some stereospecific drug action is the following: Asymmetric drug molecules enter tissues and modify the normal sequence of metabolic reactions by interacting with optically active metabolites. The interaction increases or decreases the availability of these metabolites for transport, chemical transformation, or feedback control of enzymatic reactions. The drug is an asymmetric catalyst rather than a substrate for an asymmetric macromolecular receptor. Even the example of optically active solvent may have physiological relevance, since the submicroscopic locus of drug activity may be very different from a simple aqueous solution.

A suggestive chemical analogy is provided by the procedure for the resolution of optically active hexobarbital and methylphenobarbital (4, 5). Both drugs were resolved by forming diastereoisomer pairs with (−)-methylquininium hydroxide. Each diastereroisomer pair was then resolved by fractional crystallization from alcohol-ether. In the case of hexobarbital, (−)-methylquininium (+)-hexobarbital remained in solution, while in the case of methylphenobarbital, (−)-methylquininium(−)-methylphenobarbital remained in solution. In both cases, the enantiomer most active as hypnotic remained in the solvent. The reversal of antipodal specificity in the rat might be explained as follows: An optically active metabolite, analagous in its role in this example to the (−)-methylquininium, interacts with either of the hypnotic barbiturate enantiomers with equivalent changes in its physical properties and metabolic fate.

Since "receptors" represent a largely hypothetical group of macromolecules, it would be worthwhile to extend the concept "drug-receptor interaction" to small molecule interactions as well. Many different receptor mechanisms may exist, even for the same drug. Otherwise, it is difficult to reconcile the equal activities of amphetamine isomers on the rat vas deferens (11) with the pharmacological specificity in man of the d-isomer, or the specificity of action of (−)-nicotine on guinea-pig ileum with that of (+)-nicotine in inhibiting ox red cell cholinesterase (12). The exploration of these issues has been made easier by the recent introduction of gas chromatography for the resolution of microgram amounts of optical isomers (13–17).

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The Nervous System as a Site of Action for Digitalis and Antiarrhythmic Drugs*

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Introduction

Cattell and Gold (1), in 1938, demonstrated an action of ouabain to enhance the contractile force of isolated "failed" cat papillary muscle. It has since been assumed that the important pharmacological effect of digitalis to improve the function of the failing heart is the result of an action upon the cells of the myocardium. Accepting the inotropic effects to result from an action directly on the heart, a similar site of action was ascribed to chronotropic effects produced by digitalis. There can be little doubt that in appropriate doses digitalis materials can influence the rate and rhythm of the heart, the conduction of electrical impulses within the myocardium, and increase the force of myocardial contraction by an action directly upon myocardial cells.

With the focus of attention firmly established on the myocardium, the 200 years of clinical and experimental association of digitalis-induced neurotoxicity with impending cardiotoxicity seems to have been overlooked. This association, however, is sufficiently impressive to require a careful evaluation of the nervous system as a site of digitalis action in the induction of cardiotoxicity.

In making this evaluation, the following questions must be answered:

(1) Do digitalis materials have the capacity to act directly upon the nervous system?

(2) Do alterations in neural function affect cardiac rhythm?

(3) Does alteration in neural function influence the capacity of digitalis materials to induce cardiotoxicity?

(4) Do drugs which are effective against digitalis-induced arrhythmia have the capacity to influence neural structures?

Do Digitalis Materials Have the Capacity to Act Directly upon the Nervous System?

There is considerable evidence for digitalis action directly upon neural structures. Digitalis neurotoxicity has been appreciated clinically for as long

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as the drug has been in use, and the drug can be demonstrated experimentally to act directly on the vomiting mechanism, the respiratory center, the cerebral cortex, the autonomic ganglia, and the neuromuscular junction.

Digitalis action on the nervous system has been appreciated in clinical medicine since the time of William Withering (2). Indeed, neurotoxicity has long been the harbinger of impending cardiotoxicity. In addition to the well-known symptoms of nausea, vomiting, and disturbances of color vision, Batterman and Gutner (3) list the following effects of digitalis: blurring of vision, diplopia, temporary amblyopia, scotomata, vertigo, headache, restlessness, irritability, epileptiform convulsions, hallucinations, illusions, delusions, confusion, disorientation, delirium, aphasia, stupor, and coma; in addition, trigeminal neuralgia, paresthesias, and other sensory disturbances have been observed.

Although digitalis-induced vomiting was proposed by some to result from local gastrointestinal irritation and by others to result from a reflex originating within the myocardium, the central nervous system (CNS) is now established as the primary site for this effect. The work of Hatcher and Eggleston (4) excluded the gastrointestinal tract as a site of action, since they observed digitalis-induced retching in eviscerated animals. Borison and Wang (5, 6) clearly demonstrated that ablation of the chemoreceptor trigger zone abolishes digitalis-induced vomiting. This CNS action of digitalis is of more than passing interest. Nausea and vomiting herald the onset of digitalis cardiotoxicity, an association which we believe is more than coincidental.

The respiratory center is another CNS site of digitalis action. The effect of digitalis to induce hyperpnea has been recognized for many years. A report of this effect was made by Traube (7) in 1851. Gross (8), in 1914, demonstrated a biphasic effect of strophanthidin and digitoxin on respiration in the rabbit; these drugs initially produced stimulation, and subsequently depression of respiratory activity. In his studies, Gross found respiratory depression and arrest to precede the cardiotoxic manifestations of digitoxin; with mechanical ventilation, he could maintain cardiac function in the face of otherwise lethal doses of digitoxin. Usubiaga et al (9) recently observed hyperpnea and an increase in blood pH in dogs receiving intoxicating doses of ouabain. We have observed similar ouabain-induced respiratory hyperactivity in the cat (Fig. 1). In our experiments, respiratory stimulation did not occur in animals pretreated with diphenylhydantoin, d,1-INPEA (d,1 N-isopropyl-p-nitro-phenylethanolamine), or diphenylthiohydantoin when these were administered in doses sufficient to antagonize ventricular rhythm disorders induced by digitalis (10). Usubiaga made a similar observation for lidocaine in the dog (9). In our studies, these antiarrhythmic doses of diphenylhydantoin, diphenylthiohydantoin, and d,1-INPEA usually prevented the digitalis-induced retching and vomiting which otherwise precede ventricular tachycardia (unpublished observations; c.f. also Mosey and Tyler (11)).

Perhaps the most dramatic demonstration that digitalis materials influence the CNS is found in their capacity to induce seizures. Cattell and Gold ob-

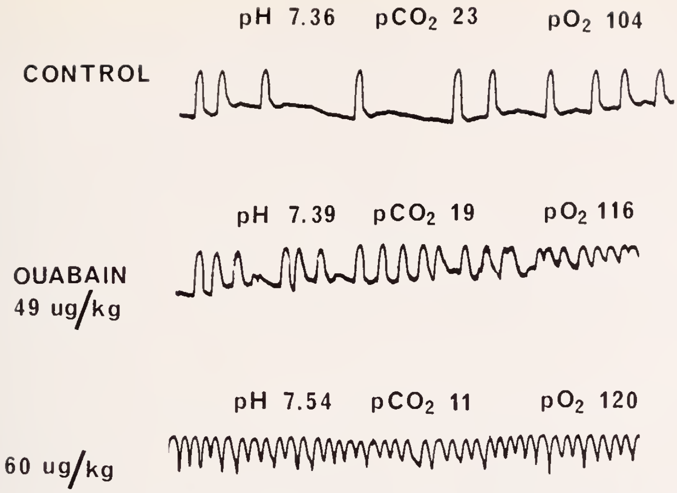

Fig. 1. *The effect of ouabain on respiration in the cat.* Two micrograms ouabain/kg/minute were infused IV in Dial-urethane anesthetized cats. Respiratory movements were recorded by means of a pneumograph. The increase in mechanical respiratory activity seen in the figure, and the resultant increase in arterial pH, pO_2, and the decrease in arterial pCO_2 parallel the increase in ventricular escape rate as demonstrated by the vagally-induced sinus arrest technique. Gillis and Standaert (unpublished observations) recently observed progressively increasing discharges in the sympathetic nerve trunks synchronous with respiration in cats receiving intoxicating doses of ouabain.

served camphor-like convulsions in unanesthetized cats given digitoxigenin intravenously; the seizures were blocked by anesthesia (12). Hatcher and Eggleston (13) observed hyperexcitability and other signs referable to excitation of the CNS in rats treated with ouabain or digitoxin. Weiss (14) reported occasional strychnine-like convulsions in cats given digitoxin. Similarly, the administration of digitalis to the unanesthetized rabbit produces tremors and convulsions (15), and digitalis-induced epileptiform convulsions have been reported to occur in man (3). These seizures are apparently initiated by an action of digitalis directly in the CNS. Gold et al (12) showed that the convulsant effect of cardiac glycosides in the rat are manifest at a time when the heart is beating strongly; convulsions are, therefore, not a terminal asphyxial event. More recently, acetylstrophanthidin and ouabain have been reported to prolong cortical afterdischarges in the cat cerebrum (16). Similarly, local application of digitalis materials to the rabbit brain produces electroencephalographic evidence of seizure activity (17).

Actions of digitalis materials on the peripheral nervous system are similarly well documented. With regard to the adrenergic nervous system, Konzett and Rothlin (18) observed that in the ouabain perfused superior cervical ganglion, electrical or chemical stimulation produced an augmented response of the nictitating membrane; higher concentrations of ouabain had an inhibitory effect. It is noteworthy that these authors found that in the absence of stimulation ouabain does not initiate activity in the nerve or the nictitating membrane; the effects of ouabain are only demonstrable in the presence of an excitatory influence. These observers also noted a correlation between the ganglionic and cardiac effects of the four glycosides tested. Perry and Reinert (19) also demonstrated a biphasic action for ouabain on the cat superior cervical ganglion-nictitating membrane preparation. Low doses of ouabain enhanced, and high doses depressed the effects of preganglionic electrical stimulation and of application of tetramethylammonium (TMA) or acetylcholine. Pretreatment of the ganglion with nicotine or hexamethonium abolished the enhancement produced by low doses of ouabain. Perry and Reinert interpreted these phenomena as an indication of a neural action of ouabain. This neural action of ouabain is not the result of cholinesterase inhibition (18, 19); ouabain is devoid of anticholinesterase activity (20).

In an effort to establish the relevance of this neural action of ouabain, Perry and Reinert extended their experiments to the isolated perfused mammalian heart. In the cat Langendorf preparation, in which cholinergic postganglionic neurones and fibers are intact, they demonstrated a ouabain enhancement of acetylcholine or TMA-induced bradycardia. In the presence of ganglionic blocking doses of either hexamethonium or nicotine, the enhancement does not occur. These results substantiated their conclusion that ouabain acts on a neural site to produce cardiac slowing. More recently, Ten Eick and Hoffman (21) observed that ouabain produces no slowing of sinus rate in the chronically denervated heart. We (22) have similarly observed the failure of digitalis to induce sinus slowing in bilaterally vagotamized cats.

Another demonstration of ouabain action in sympathetic ganglia was provided by Birks (23), who reported a tenfold increase in the output of acetylcholine from perfused sympathetic ganglia after ouabain was added to the perfusate. The ouabain effect was biphasic; it increased to a maximum, and despite continued stimulation, declined. In like manner, Ten Eick and Hoffman (24) recently demonstrated a ouabain-induced enhancement of electrical activity in preganglionic adrenergic nerve fibers of the cat stellate ganglion and the cardiac vagus. These observers noted that the administration of ouabain augments the electrical response of postganglionic fibers to submaximal preganglionic stimuli; the augmented response indicates a drug-induced recruitment of responding nerve fibers. In addition to monitoring the electrical activity in the nerve, these investigators also recorded contractions of the nictitating membrane. As anticipated from the electrical data, they observed augmented contractions in ouabain-treated preparations, and demonstrated these to result from an excitatory action of ouabain on both pre- and post-ganglionic fibers of the superior cervical ganglion. These authors found no inotropic effect of ouabain on the nictitating membrane.

Finally, digitalis has been shown to affect the nerves to skeletal muscle. Birks (23) made the following observations which he interpreted as indicating a pre-synaptic action of ouabain: First, innervated frog sartorius muscle is induced to spontaneous twitching in the presence of ouabain and this twitching is abolished by d-tubocurarine. Second, in the presence of ouabain, the frequency of miniature end plate potentials increased from 1/second to 100/second. This increase in frequency is associated with an increase in amplitude.

Recently, we (unpublished observations) have observed ouabain-induced enhancement of post-tetanic repetitive discharges in the cat soleus motor axons; the increased neural activity causes a corresponding enhancement of potentiation of soleus muscle (Fig. 2). These effects of ouabain are abolished by the administration of antiarrhythmic doses of quinidine or diphenylhydantoin. As noted above, the facilitatory actions of ouabain cannot be explained on the basis of an anticholinesterase action because the drug is not an inhibitor of cholinesterase (20).

Thus, the answer to the question, "Do digitalis materials have the capacity to act directly upon the nervous system?," is clear. Those investigators who have sought the neural effects of digitalis materials have found them at all levels of the neuraxis.

Do Alterations in Neural Function Affect Cardiac Rhythm?

There is considerable experimental evidence to indicate that alterations in neural function can induce or prevent cardiac rhythm disorders. Hockman, Mauck, and Hoff (25) induced bigemini, trigemini, ventricular tachycardia, and ventricular fibrillation by stimulation of the diencephalic and mesencephalic reticular formation. These rhythm disturbances reverted promptly to regular sinus rhythm with cessation of electrical stimulation. Mauck, Hockman, and Hoff (26) also demonstrated that an aberrant cardiac rhythm, which bears a striking similarity to the Wolff-Parkinson-White complex in man, can be produced experimentally in normal healthy dogs by electrical stimulation of the mesencephalic reticular formation. Similarly, Purpura et al (27) demonstrated the induction of cardiac irregularities by selective stimulation of the reticular formation, and observed blockade of these irregularities by small doses of barbiturates and other neurodepressant drugs. Richard-

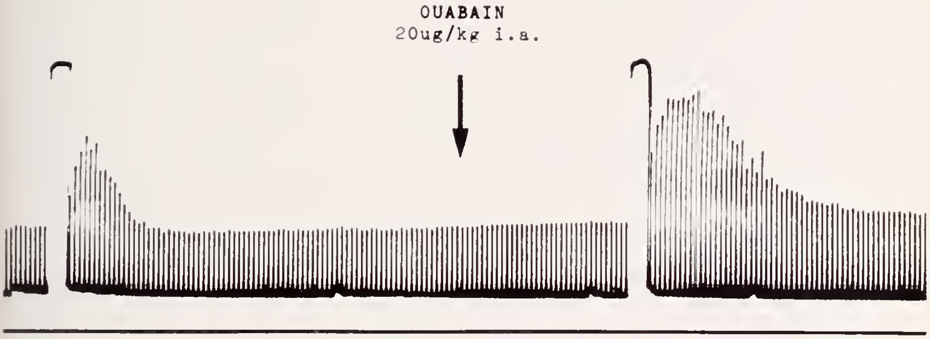

FIG. 2. *Ouabain augmentation of post-tetanic potentiation in the cat soleus neuromuscular junction.* On the left, the record shows control soleus muscle contractile responses to supramaximal stimuli applied to the soleus nerve at intervals of 1 every 2.5 seconds. The interposition of a tetanic stimulus (400 Hz for 10 seconds) leads to the development of post-tetanic potentiation. After intra-arterial administration of ouabain, post-tetanic potentiation is increased in magnitude and duration. The ouabain-induced enhancement is produced by exaggerated post-tetanic neural discharges originating in the motor nerve endings (for details of method see references 51, 54, 74, and 75).

son et al (28), studying both dogs and man, abolished cardiac arrhythmias induced by electroconvulsive therapy with atropine. Manning and Cotton (29) demonstrated that the simultaneous activation of sympathetic and parasympathetic innervation of the heart results in the induction of cardiac rhythm disorders.

The action of drugs on neural structures has also been shown to influence cardiac rhythm. Local application of deslanoside to the floor of the fourth ventricle of the brain initiates ventricular tachycardia which is prevented by spinal cord transection and vagotomy (30). Cardiac irregularities are also observed following the application of other neuroexcitatory substances, such as strychnine and picrotoxin (30). Hockman, Mauck, and Hoff (31) have demonstrated abolition of centrally induced ventricular arrhythmias by the administration of diphenylhydantoin. We have confirmed these findings (unpublished observations) and have noted that cardiac rhythm disorders produced by electrical stimulation of the subthalamic nucleus of the cat are also abolished by d,1-INPEA and propranolol.

Thus, the answer to the question, "Do alterations in neural function affect cardiac rhythm?," is clear. The activation of certain neural pathways regularly produces cardiac arrhythmias, and depression of these pathways regularly abolishes them.

Does Alteration in Neural Function Influence the Capacity of Digitalis Materials to Induce Cardiotoxicity?

In 1964, Erlij and Mendez (32) found that exclusion of the adrenergic nervous system by chronic sympathectomy increases the dose of digitoxin required to produce fatal ventricular rhythm disorders. Boyajy and Nash (33) found that spinal section and spinal anesthesia produce similar results. Wilson, Paradise, and Stoelting (34) found that epidural block with lidocaine results in comparable protection. More recently, we (35) demonstrated that although spinal cord transection affords remarkable protection against ouabain-induced ventricular rhythm disorders in some cats, this procedure failed to protect other animals. In those animals in which spinal cord transection results in a substantial reduction in heart rate and blood pressure, the dose of ouabain required to produce ventricular rhythm disorders is greatly increased. No protection against ouabain-induced ventricular rhythm disorders is observed in animals whose heart rates and blood pressures are uninfluenced by spinal cord transection; therefore, the capacity of spinal cord transection to increase the dose of ouabain required to produce ventricular arrhythmia seems to be related to the capacity of this surgical procedure to depress adrenergic neural influence on the heart.

Additional evidence to support the participation of the nervous system in the cardiac actions of digitalis can be found in the work of Schaal et al (36). These investigators found that in chronically denervated dogs, digitalis failed to prolong the functional atrioventricular nodal refractory period, a characteristic action of digitalis prior to denervation. Moreover, the tolerance of these animals to the cardiotoxic effect of digitalis materials is increased (37). Interestingly, the inotropic action of ouabain is not changed in the extrinsically denervated dog heart (38). This finding is in agreement with those of Cairoli, Reilly, and Roberts (39), who found that the positive inotropic action of ouabain on the cat papillary muscle was not influenced by reserpine pretreatment. However, in six baboons, Willman et al (40) reported ouabain cardiotoxicity to be uninfluenced by cardiac autotransplantation; it is difficult to evaluate the results of these workers because they indicated neither route nor rate of drug administration, nor the endpoint for drug toxicity used.

The contention that the susceptibility of the myocardium to arrhythmia induced by digitalis is at least partially a function of adrenergic neural activity in the heart is further supported by the following pharmacologic data: Reserpine pretreatment increases the dose of digitalis materials required to produce ventricular tachycardia or death (32, 41–45). Although Morrow, Gaffney, and Braunwald (46) reported that reserpine pretreatment or chronic cardiac denervation did not influence the dose of ouabain necessary to produce three consecutive extrasystoles, that endpoint is different from that employed by most other investigators. In a recent review, Mason and Braunwald (47) point out that reduction in adrenergic influence on the heart diminishes the incidence of digitalis-induced ventricular arrhythmias. Similarly, adrenergic neuronal depression with beta TM-10 (41) and guanethidine (22) increase the dose of digitalis required to induce cardiac rhythm disorders.

These findings of important neural contributions to the suppression of digitalis-induced ventricular rhythm disorders find further support in the failure of antiarrhythmic drugs to produce their expected effects in the presence of an already depressed nervous system. Pronethalol and quinidine fail to influence acetylstrophanthidin-induced ventricular tachycardia in animals pretreated with reserpine (42). In spinal animals with depressed adrenergic tone, diphenylhydantoin loses its ability to protect against ouabain-induced cardiotoxicity (48). Interestingly, diphenylhydantoin does not produce its characteristic bradycardia in cats pretreated with pronethalol even though the bradycardia produced by diphenylhydantoin is not related to blockade of beta adrenergic receptors (49). Recently, Flacke, Osgood, and Bendixen (50) have shown that exclusion of autonomic influence on the heart by epidural anesthesia and atropine prevents propranolol-induced changes in cardiovascular function.

Thus, the answer to the question, "Does alteration in neural function influence the capacity of digitalis materials to induce cardiotoxicity?," is clear. Surgical or pharmacologic reduction in neural activity diminishes the capacity of digitalis materials to disorganize the heart.

Do Drugs Which Are Effective Against Digitalis-Induced Arrhythmia Have the Capacity to Influence Neural Structures?

Neurodepression is a common property of many antiarrhythmic drugs (51, 52). Depression of the nervous system by these drugs expresses itself as anticonvulsant actions, alteration of spinal cord reflex activity, local anesthetic effects, and reduction in repetitive firing of peripheral nerves.

Harris and Kokernot (53) were the first to note a relationship between anticonvulsant and antiarrhythmic drug action. They demonstrated the efficacy of diphenylhydantoin and phenobarbital in suppressing arrhythmias in dogs with coronary arteries ligated. The first demonstration of the effectiveness of an anticonvulsant in suppressing arrhythmias induced by digitalis was provided by Mosey and Tyler (11), who observed reduction in digitalis-induced cardiotoxicity by diphenylhydantoin. This correlation between anticonvulsant and antiarrhythmic activity has been reaffirmed repeatedly. Diphenylthiohydantoin, an effective antiarrhythmic drug (10), has been shown to antagonize maximal electroshock seizures in rats (54, 55). Quinidine and procaine amide, the prototype antiarrhythmic agents, have been observed to suppress maximal electroshock seizures in the rat (56). Similarly, other drugs well-known for their antiarrhythmic actions are effective anticonvulsants. Procaine, lidocaine, and other local anesthetics depress seizure activity in the cat, monkey (57), and the mouse (58). Propranolol and pronethalol are effective in elevating seizure threshold and modifying maximal electroshock seizures in rats (59), as well as suppressing the convulsant effects of nicotine and strychnine (60) in rats and mice (61).

Antiarrhythmic drugs have also been shown to depress reflex activity in the spinal cord. The iontophoretic application of procaine to interneurones, motoneurones, and Renshaw cells of the cat spinal cord depresses spike potentials in all these structures (62). Diphenylhydantoin reduces polysynaptic discharges in the cat spinal cord; it depresses the transmission of repetitive trains of impulses through the monosynaptic pathway, and it markedly diminishes post-tetanic potentiation (63). Significantly, the drug also reduces the amplitude of maximal responses of postganglionic sympathetic neurones to stimulation of preganglionic fibers of the cat stellate ganglion (63); as in the spinal cord, diphenylhydantoin diminishes transmission of high frequency trains through the ganglion, and post-tetanic potentiation is similarly depressed. Evidence for a spinal cord depressant action of pronethalol has recently been provided by the demonstration that the drug diminishes polysynaptic flexor responses in guinea pigs and rabbits (64, 65).

Recently, attention has been focused on the correlation between antiarrhythmic activity and what is called "local anesthetic activity" (66, 67). The latter has been determined in many ways. Dawes, in his classic paper (68), used the guinea pig wheal test to demonstrate local anesthetic activity for a large number of synthetic substitutes for quinidine. Local anesthetic properties of procaine, procaine amide, and lidocaine have been found in too many systems to enumerate. Schmidt and Hanna (69) have recently demonstrated that quinidine and pronethalol act as local anesthetics in the earthworm and the rabbit corneal anesthesia tests. Levy (70), who also used the rabbit corneal anesthesia method, was able to demonstrate local anesthetic activity for pronethalol, propranolol, and d-propranolol. Gill and Vaughan-Williams (71), using the guinea pig wheal test, observed local anesthetic action for pronethalol and propranolol, and Paradise (66), using the rat intradermal wheal test, demonstrated local anesthetic effects for d,1-INPEA. It is obvious from the foregoing that "local anesthetic effects" are defined to suit the convenience of many authors. Furthermore, it is obvious that the term has become dissociated from its original clinical meaning. The present loose use of the term has occasioned several difficulties; since the words "local anesthetic" are historically associated with the drugs used clinically to produce local anesthesia, the reader automatically associates them with the actions of procaine and assumes a common mechanism of action for all drugs described as local anesthetics. Unfortunately, there is no basis for this assumption. In most cases, the mechanism of action is unknown and, as presently used, the term "local anesthetic activity" implies little more than a drug-induced disturbance in the function of some excitable membranes. Furthermore, although in practice the assay usually is based on a measure of neural function, many authors have no reluctance to extrapolate their results to the myocardial cell. The resulting confusion adds little to our knowledge of antiarrhythmic drug action. It should not be overlooked, however, that deplorable as the term "local anesthetic activity" and its use have become, these experiments clearly show that many antiarrhythmic drugs have the capacity to interfere with neural function.

Perhaps the ultimate irony is that the term "local anesthetic" has become so ill-defined that many authors feel no compunctions in translating the action of a drug to interfere with function in neural tissues to mean proof of nonspecific or general myocardial depression.

The notion of local anesthetic activity has been most recently used to explain away the discrepancy between the effectiveness of certain drugs in blocking the beta adrenergic receptors, and their effectiveness to antagonize the digitalis-induced arrhythmia (67, 72). The early observation of the protective effect of reserpine and certain beta adrenergic blocking drugs (for example, pronethalol) against ventricular rhythm disorders induced by digitalis called attention to the possibility that catecholamines and their blockade may play a role in the induction and suppression of the arrhythmias. However, recent work (51, 52, 66, 72, 73) clearly indicates that beta adrenergic blocking properties for pronethalol, propranolol, and INPEA are independent pharmacologic properties and do not play a role in the blockade of cardiac arrhythmias induced by digitalis.

While some investigators propose direct actions of pronethalol, d-pronethalol, propranolol, and d-propranolol on the myocardium as the sole explanation for the blockade of digitalis-induced ventricular rhythm disorders, we (10, 51, 52) proposed that the action of these drugs is related to their neurodepressant properties. In order to dissociate peripheral neural depression from blockade of beta receptors, we assayed neural depression in a nonadrenergic neuroeffector system. We found that pronethalol, d-pronethalol, propranolol, d-propranolol, d,1-INPEA, d, INPEA, 1, INPEA, diphenylhydantoin, diphenylthiohydantoin, procaine, and lidocaine all depress repetitive afterdischarge originating in the nerve terminals of the soleus nerve, as well as the resultant post-tetanic potentiation of the soleus muscle (51, 54, 74, 75). We have recently observed similar effects for the antidigitalis materials quinidine, guanethidine, and beta TM-10 (unpublished observations). It is interesting and important to note that the doses which depress neurorepetitive afterdischarges are identical with the antidigitalis doses for these drugs in the cat.

Thus, the answer to the question "Do drugs which are effective against digitalis-induced arrhythmia have the capacity to influence neural structures?," is clear. Virtually all the drugs reported to antagonize arrhythmia induced by digitalis have also been observed to be capable of interfering in some way with neural function. Furthermore, there is an explanation which we have proposed for the role of the autonomic nervous system in the regulation of cardiac rhythm. As we (76) suggested, "It is generally recognized that the heart is richly innervated with autonomic fibers and that these fibers can influence automaticity, conductivity and excitability. A consideration of the anatomy of the heart indicates that autonomic influence must be mediated through a multiplicity of individual neuroeffector synapses, and that each nerve terminal influences its immediate region of the myocardium. Under normal circumstances, each nerve terminal speeds or slows progression of the cardiac impulse through its domain in concert with the activity of adjacent terminals. Under experimental conditions of synchronous stimulation of extracardiac nerve trunks, the response of the nerve terminals and the underlying myocardium is perforce uniform, and a gross slowing or speeding of the heart results. Insofar as each axon or nerve terminal has the capacity for independent activity, the system is potentially capable of becoming discoordinated. Discordant neural activity would result in fractionation of the myocardium, i.e. conductivity, automaticity and excitability would vary from region to region, and the flow of electrical activity through such a fractionated myocardium will become turbulent (77). The evidence suggests that digitalis affects the nervous system to produce focal changes in cardiac autonomic nerve function. This suggestion is in accord with the generally recognized principle that a given dose of drug will influence members of a population to different degrees. Thus, nonuniformity in the neural response to digitalis may result in disorganized cardiac activity. The action of drugs to depress digitalis-induced arrhythmia may result from their capacity to maintain or restore organized neural control."

Conclusion and Summary

From the foregoing, it is clear that neural sites are potentially capable of playing a major role in the induction of arrhythmias by digitalis, and their suppression by antiarrhythmic drugs. As we have pointed out, digitalis does exert effects directly on the myocardium, and this is also true for quinidine, pronethalol, propranolol, and diphenylhydantoin. Furthermore, it is reasonable to expect that drugs which influence the excitable membranes of nerve cells will similarly influence the excitable membranes of muscle cells. As we (51, 52) have previously stated, the capacity of a drug to excite or depress either neural or myocardial cells will depend upon the properties of the tissues, the characteristics of the drug, and the dose employed. Differences such as the larger surface area to volume ratio in the case of terminal branches of nerves, as compared with myocardial muscle fibers, should make some drug effects more prominent in one tissue than in the other. It is well-known that fibers of small diameter are generally more sensitive to blockade by local anesthetics than are fibers of large diameter, and that higher concentrations of local anesthetics are required to block conduction in large diameter fibers. Thus, still higher concentrations might be needed to depress electrical activity in myocardial fibers devoid of neural tissue. It might be possible for some drugs to antagonize digitalis-induced ventricular rhythm disorders without causing appreciable myocardial depression, whereas with others, the antiarrhythmic action may be invariably associated with significant myocardial depression. Since physiologic or pharmacologic alteration in the functional activity of both the central and peripheral nervous system can produce striking changes in the function of the heart, first the search for new antiarrhythmic drugs should include agents with demonstrated neurodepressant properties, second, the nervous system must be considered as a possible source for the initiation of cardiac rhythm disorders in the clinical setting, and finally, alterations in the state of the nervous system may play a vital role in the management of patients with cardiovascular disease (78, 79).

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Atrial Fibrillation and Mitral Regurgitation

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Different investigators (1-9) have described changes in the atrial pressure curve, mainly in the left atrial tracing, in the presence of atrial fibrillation. In addition to the absence of A waves, a well-known finding is the existence of a systolic plateau due to a decrease or abolition of the X depression in the atrial curve. These findings were first noted in pressure tracings of the right atrium.

Their similarity to the changes observed in tricuspid regurgitation led several authors (7, 8) to admit that this arrhythmia could enhance or provoke systolic regurgitation. Coelho and Amram (10, 11) have studied this problem both in patients and in experimental animals, and have concluded that atrial fibrillation accentuates a preexisting tricuspid insufficiency. These authors point out, however, that the absence of X descent in the atrial curve should not be necessarily interpreted as indicative of tricuspid regurgitation, and that it is only when the right atrium is large and there exists myocardial failure that atrial fibrillation leads to valvular insufficiency. They also found that X descent can be seen when the right atrial pressure is not high. Braunwald et al (3) have shown that with atrial fibrillation, the longer the diastolic periods, the more marked is the X descent in the left atrial pressure tracing, a finding which is not in accordance with the existence of valvular insufficiency.

In a patient with pure mitral stenosis that we have studied by left heart catheterization (transeptal and retrograde arterial), paroxysmal atrial fibrillation developed during the procedure. As can be seen in Figure 1, the configuration of the left atrial pressure tracing changed. The systolic descent X between waves C and V was substituted by a systolic plateau C + V; on the third curve, which follows a longer diastole, an X descent can be seen. In Figure 2, on the right, it can be seen that the injection of indicator dye (carboindocyanine) into the left ventricle through the retrograde arterial catheter was not followed by early appearance of dye in the left atrium, as could be demonstrated by drawing blood from this chamber through the transeptal catheter. This way, we think we have demonstrated in this case that the plateau configuration of the left atrial pressure curve that appeared when the rhythm changed to atrial fibrillation was not due to mitral regurgitation.

In a sizeable number of cases of pure mitral stenosis and atrial fibrillation, we demonstrated the absence of reflux to the left atrium by the injection of indicator dye into the left ventricle.

In previous papers (6, 10, 11, 12) we have called attention to the fact that patients with pure mitral stenosis with atrial fibrillation, undergoing comissurotomy, have no evidence of reflux at the time of surgery.

It is not quite clear why the X descent in the atrial curve is absent or diminished when there is atrial fibrillation. Ankeney, Fishman, and Fritts (13), and

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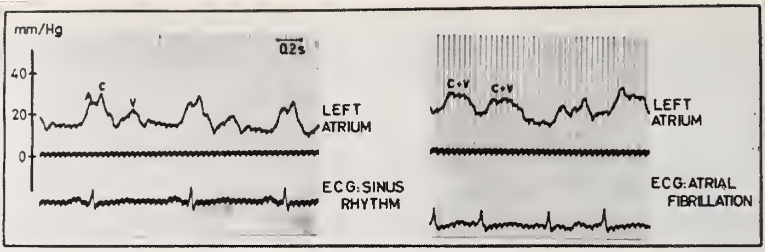

FIG. 1. Left atrial pressure curves in a patient with pure mitral stenosis, who developed paroxysmal atrial fibrillation during atrial transeptal catheterization. On the left is shown an atrial tracing with an X descent, and V waves that are smaller than A and C waves; On the right, during atrial fibrillation, the pressure tracing shows a systolic plateau.

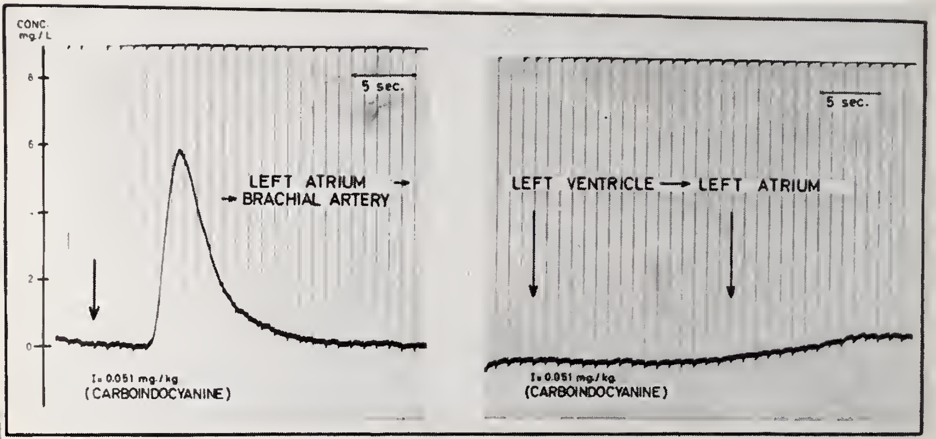

FIG. 2. Dye dilution curves in a case of mitral stenosis, during an episode of atrial fibrillation that occurred during a hemodynamic study. On the left is reproduced the peripheral arterial curve, which has a normal configuration. On the right, it can be seen that after injection of dye into the left ventricle through a retrograde arterial catheter, there is no early appearance of the indicator in the left atrium.

Nixon and Pollis (14), for instance, attribute it to the lack of atrial relaxation in fibrillation; on the other hand, Gordon, Kirschner, and Moscovitz (15) considered the possibility that a distended and fibrillating atrium shows a change of its pattern of response to the changes in pressure, an opinion with which we agree.

The likely occurrence of regurgitation during the early ventricular systole, when there is atrial fibrillation, has been related to the role that, according to some authors (16, 17), atrial contraction seems to play in the closure of the atrioventricular valves. Little (17) studied the subject in relation to the closing of the tricuspid valve, and showed that atrial contraction leads the valves to a semi-closing position that ventricular contraction completes. On the other hand, the studies of Skinner, Mitchell, and Wallace (18) have shown that the mitral valve can close exclusively as a result of atrial activity. These authors believe that mitral regurgitation is an important factor in the genesis of the changes in the atrial pressure tracing that occur with atrial fibrillation. They have also demonstrated, experimentally, that the temporal relationship of atrial contraction, in relation to the ventricular one, is important in producing regurgitation. We think that the Skinner experiments are not conclusive because they were carried out on dogs with auriculoventricular block, under the effect of ganglioplegic agents, and therefore with an altered ventricular activation which may have influenced the results.

The fact that according to some authors (18–22) valvular regurgitation can exist in some ectopic rhythms not preceeded by atrial contraction, does not prove that the absence of atrial contraction is responsible for that regurgitation, and does not allow the conclusion that there is necessarily valvular regurgitation in atrial fibrillation due to the lack of a coordinated atrial contraction. Other studies (23–25) have shown that atrial contraction need not occur for effective closure of the mitral valve to take place.

Figure 3 reproduces a left atrial pressure curve from a case of pure mitral stenosis, obtained during an episode of septal rhythm and during sinus rhythm. The typical configuration of valvular insufficiency with giant, wide V waves, with a rapid ascent during septal rhythm, changes completely when the rhythm is of sinus origin. It is interesting to note that in the fourth, fifth, and sixth pulsations, where the atrial contraction is progressively further from the ventricular contraction, the typical aspect of mitral regurgitation is progressively less evident. In figure 4, the left atrial pressure curve of a case of mitral stenosis during a period of auricular rhythm is reproduced; one can see the characteristic aspect of valvular insufficiency.

Multiple episodes of atrial fibrillation were provoked experimentally in six dogs by the application of methacholine directly over the left atrial appendage, and we could observe the following changes in all of them: (1) elevation of the left atrial mean pressure in relation to the end-diastolic ventricular pressure;

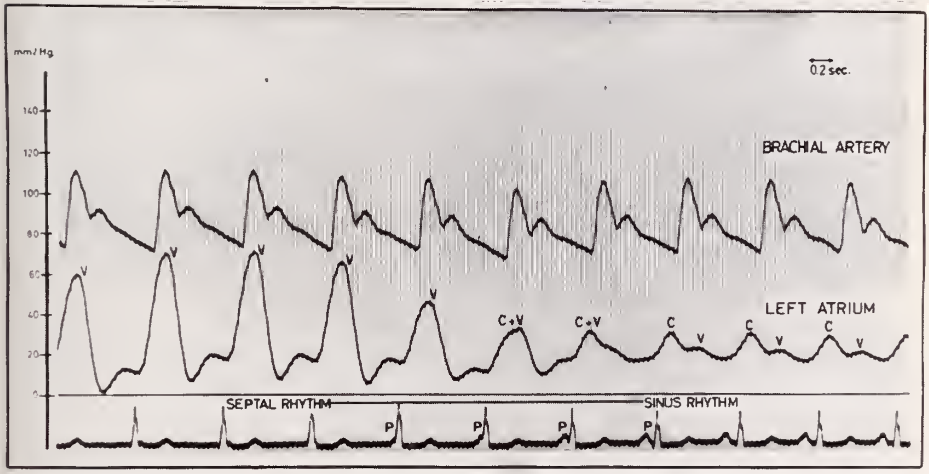

Fig. 3. Left atrial pressure tracing recorded during an episode of septal rhythm and the transition to regular sinus rhythm. During the ectopic rhythm, the left atrial pressure curve has the typical configuration of mitral regurgitation.

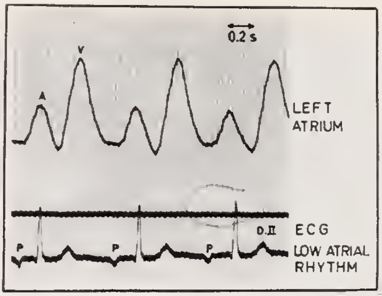
0.2 s
V
A
LEFT ATRIUM
D.II ECG
P
LOW ATRIAL RHYTHM

Fig. 4. Left atrial pressure curve of a case of mitral stenosis, recorded during an episode of atrial rhythm. The configuration is typical of mitral regurgitation (prominent V waves and steep Y descent).

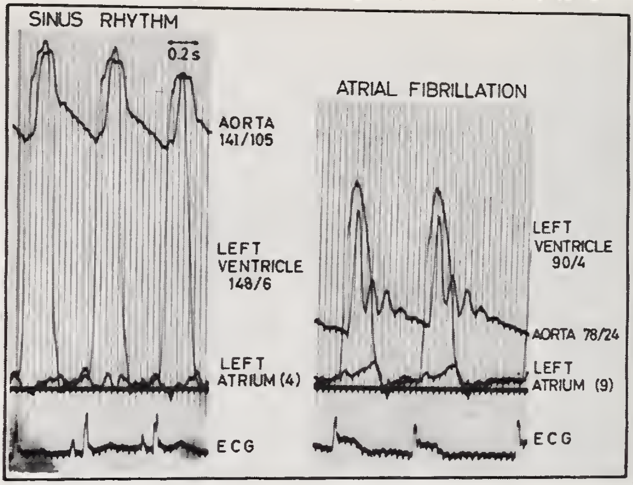
SINUS RHYTHM
0.2 s
AORTA 141/105
LEFT VENTRICLE 148/6
LEFT ATRIUM (4)
ECG
ATRIAL FIBRILLATION
LEFT VENTRICLE 90/4
AORTA 78/24
LEFT ATRIUM (9)
ECG

Fig. 5. Left atrial, ventricular, and aortic pressure tracing in a dog with atrial fibrillation induced by methacholine. On the left, we can see the pressure curves during sinus rhythm, and on the right, during atrial fibrillation. The left ventricular systolic pressure dropped from 148 to 90 mm Hg, and the left atrial mean pressure rose from 4 to 9 mm Hg. The cardiac index diminished from 115 to 98 cc/kg/minute.

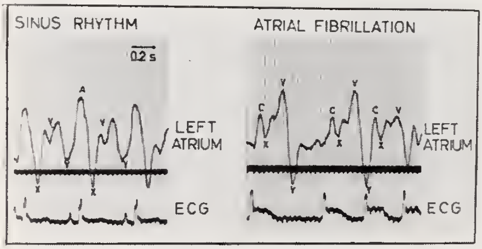
SINUS RHYTHM
0.2 s
A
V
X
LEFT ATRIUM
ECG
ATRIAL FIBRILLATION
C
V
X
LEFT ATRIUM
ECG

Fig. 6. Left atrial pressure tracing in the same experiment reproduced in Figure 5, recorded at a higher sensitivity. During atrial fibrillation A waves are absent, the X descent is markedly diminished, and the V waves are higher.

(2) a fall of the systolic ventricular and arterial pressure; (3) a variable decrease in the cardiac output; and (4) a change in configuration of the atrial pressure curve, suggestive, according to some authors, of mitral regurgitation.

In order to clarify this point we have also utilized in these experiments a study of dye dilution curves. In none of the experiments (20 on six dogs) did the curves demonstrate regurgitation during fibrillation.

The results obtained in one of the experiments are reproducen in Figures 5 and 6. We can see that the left atrial mean pressure rose from 4 to 9 mm Hg

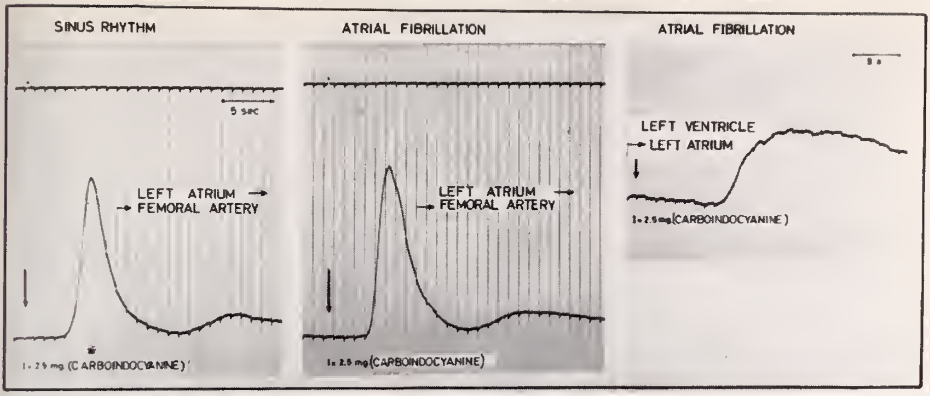

FIG. 7. Dye dilution curves in the same experiment (Figs. 5 and 6). The peripheral arterial curves recorded during sinus rhythm and atrial fibrillation are both normal. On the right, it can be seen that the injection of dye into the left ventricle was followed by its appearance in the left atrium only 8 seconds later, therefore excluding mitral regurgitation.

while the ventricular systolic pressure decreased from 148 to 90 mm Hg, with a slight variation in the end-diastolic pressure. The cardiac index decreased from 115 to 98 cc/kg/minute. The left atrial pressure curve changed considerably; A waves were not present, and there was marked diminution of the X depression and accentuation of the V waves. Dye dilution curves obtained in this same experiment during sinus rhythm and atrial fibrillation, are shown in Figure 7. It can be seen that the curves have an identical configuration, and that the injection of dye into the left ventricle was followed by its appearance in the left atrium only 8 seconds later, therefore excluding mitral regurgitation.

Daley and colleagues (7) have also utilized dye dilution studies in experimental atrial fibrillation, and they claim to have demonstrated valvular regurgitation in their experiments.

Gould and co-workers (26) used a technique of injection of ascorbic acid into the right ventricle to be detected in the right atrium, and found out that atrial fibrillation does not invariably lead to valvular insufficiency.

Several investigators (27–29), using left heart indicator dilution curves in man and experimental animals, as well as ourselves, could not confirm the existence of mitral regurgitation during atrial fibrillation.

Left ventricular angiocardiographic studies (23, 24, 30), have not disclosed left atrial opacification in a number of patients with atrial fibrillation.

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Interaction of Calcium, Norepinephrine, and Bretylium in Antagonizing Myocardial Depression Caused by Antiarrhythmic Drugs*

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In studies designed to evaluate and compare quantitatively the antifibrillatory properties of the major antiarrhythmic drugs by measuring their effect on electrical ventricular fibrillation threshold (1), parallel studies of their effect on mechanical performance and rate were also carried out. The drugs investigated included quinidine, procaine amide, lidocaine, diphenylhydantoin, propranolol, and bretylium.

This report will describe our observations on the effects of these agents on the mechanical performance of the totally isolated blood perfused dog heart under conditions of controlled constant loading, outflow resistance, heart rate, and coronary perfusion pressure (2, 3). With this preparation, left ventricular diastolic volume, as well as stroke volume and left ventricular pressure, could be measured with nervous and systemic vascular influences excluded.

Although the depressant effects of quinidine and procaine amide on mechanical performance are well known (4, 5), the effects of drugs which have been more recently introduced into antiarrhythmic therapy (lidocaine, diphenylhydantoin propranolol, and bretylium) have not been as thoroughly explored. With the exception of bretylium (which was positively inotropic), all the other drugs tested were found to decrease the strength of contraction of the isolated heart. As bretylium was also found to have the most clear-cut, measurable antifibrillatory action among these drugs, we explored a possible relationship between the antifibrillatory action of a drug and its effect on mechanical performance. Myocardial depression induced by antiarrhythmic agents was antagonized with positive inotropic agents such as calcium, norepinephrine, and bretylium. The effects and interactions of these agents on the heart were of interest, and some of the observations are included in this report.

Drug Effect on Mechanical Performance

Mechanical performance was assessed in the isolated blood supported heart removed from the chest. Left ventricular volumes and pressures were recorded from a fluid filled balloon in the left ventricle which ejected to a "prosthetic dog arrangement" attached by rigid large bore tubing to a plethysmograph. Filling pressure, outflow resistance, and coronary blood flow were set up and held constant independently of each other. The heart ordinarily beat spontaneously, but in many experiments the His bundle was ligated and the heart paced electrically to exclude the influence of changes in rate on contractility. The myo-

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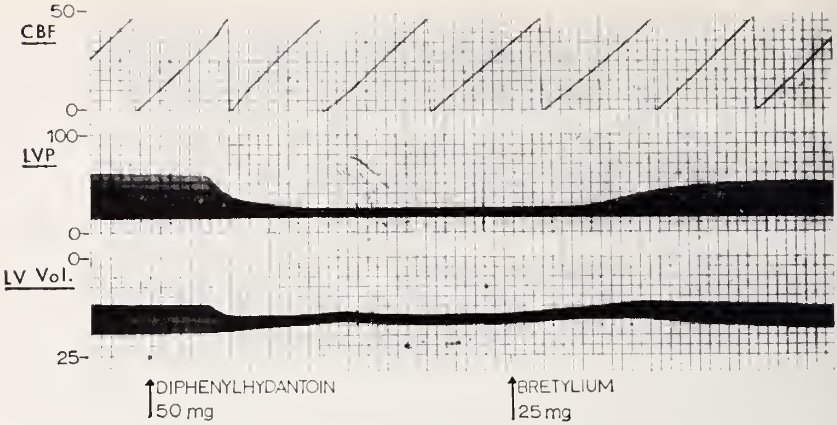

Fig. 1. CBF = coronary blood flow (ml/minute given by slope); LVP = left ventricular pressure (mm Hg); and LV Vol = left ventricular volume (ml). Time between arrows = 3 minutes (courtesy of American Journal of Cardiology).

cardium was perfused with a cannula in the aorta positioned just distal to the coronary ostia which supplied oxygenated blood from a separate support system. Coronary perfusion pressure was set at a constant level which was maintained independent of any change in coronary artery resistance. All drugs were injected into the coronary perfusion circuit. This preparation has been previously described in detail (2, 3).

It was observed that all of the agents tested (except bretylium) lowered left ventricular stroke volume and pressure when given in the usual therapeutic dose range. These observations confirmed the previously reported myocardial depressant effects of quinidine (4), procaine amide (5), lidocaine (6), propranolol (7), and diphenylhydantoin (8).

The mechanical response to diphenylhydantoin (50 mg) injected into the coronary perfusion circuit may be seen in Figure 1. The fall in stroke volume and pressure of the left ventricle was associated with a marked decrease in diastolic volume. Since filling pressure and outflow resistance were constant, the decrease in diastolic volume represented decreased diastolic distensibility (myocardial rigor). There was also decline in coronary blood flow from 60 to 48 ml/minute (change in slope) while coronary perfusion pressure remained constant, which indicated that diphenylhydantoin increased coronary vascular resistance. The subsequent administration of 25 mg of bretylium antagonized all of these actions.

Propranolol, given in relatively low doses (0.1–0.15 mg/kg), produced the most severe and refractory myocardial depression that was observed with any of the drugs tested. Figure 2 shows a typical example of the effect of propranolol on the mechanical performance of the isolated heart. In this experiment, the His bundle was ligated and the heart paced electrically to prevent bradycardia induced by propanolol. The injection of propranolol (2 mg) caused acute cardiac dilatation, as diastolic volume increased from 19 to 27 ml, and stroke volume declined from 14 to 5 ml per stroke. The subsequent injection of bretylium (50

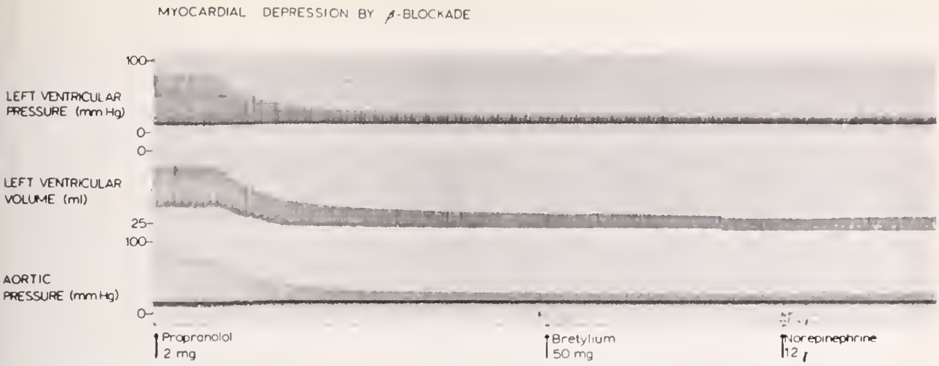

Fig. 2. See text.

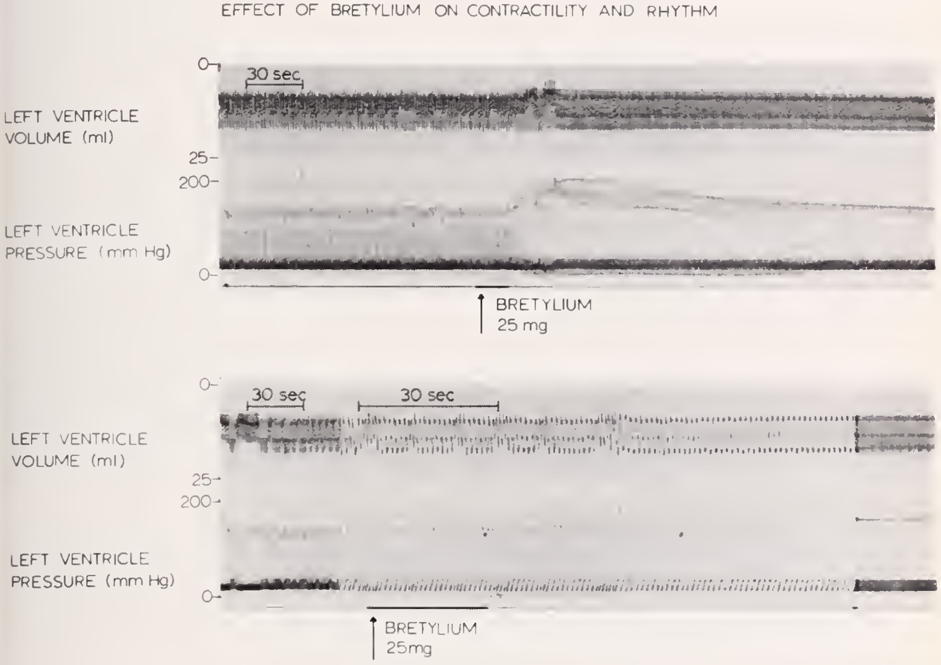

Fig. 3. See text.

mg) and norepinephrine (12 μg) had almost no effect after adrenergic blockade with propranolol. In contrast, myocardial depression produced by quinidine, procaine amide, lidocaine, and diphenylhydantoin was easily antagonized by much smaller doses of bretylium or norepinephrine.

In distinct contrast to the other antiarrhythmic agents, bretylium consistently augmented myocardial contractile strength without altering the sinus rate in the spontaneous beating isolated heart. Figure 3 shows a typical example of both the positive inotropic and antiarrhythmic effects of bretylium. The heart was beating spontaneously, but the contractions were erratic because frequent ventricular extrasystoles were occurring. The injection of bretylium (25 mg) was followed by a progressive rise in the force of contraction to a peak, followed by a decline to a lower but still increased level. The arrhythmia may be observed to disappear approximately 20 seconds after the force of contraction has reached a peak (upper panel). The lower panel shows the reappearance of the arrhythmia 15 minutes after the blood level of bretylium was lowered by replacing the blood in the perfusion reservoir with blood that contained no bretylium. After another injection of bretylium (25 mg), there was a rise in stroke volume and left ventricular pressure, which can be observed to rise further after the arrhythmia has been eliminated. The high speed section of the record shows the rapid and abrupt elimination of the arrhythmia after bretylium was given.

Bretylium (25–50 mg) also antagonized the myocardial depressing effect of quinidine, procaine amide, diphenylhydantoin, and xylocaine. Figure 1 illustrates the positive inotropic effect of bretylium (25 mg), which antagonized the myocardial depression induced by diphenylhydantoin. An increase in diastolic volume (distensibility) toward normal, as well as an increase in stroke volume, followed the injection of 25 mg of bretylium.

The Effect of Antiarrhythmic Agents on Ventricular Fibrillation

Previous studies of the antifibrillatory actions showed that only bretylium and propranolol elevated ventricular fibrillation threshold. Quinidine, procaine amide, and lidocaine were without measurable effect, while diphenylhydantoin actually depressed fibrillation threshold below control values (1).

Bretylium was found to have marked antifibrillatory action, elevating threshold from 2 to 6 times the control value. Moreover, bretylium regularly induced a phenomenon of spontaneous defibrillation whereby ventricular fibrillation triggered by high current shocks reverted to sinus rhythm spontaneously after fibrillating for periods lasting up to 2 minutes. In contrast, the untreated heart had to be electrically defibrillated once ventricular fibrillation lasting longer than ½ second was induced (1, 9).

Propranolol had considerably less antifibrillatory action, both in the degree to which fibrillation threshold was elevated, as well as in the inconsistency of its antifibrillatory effect. Propranolol increased fibrillation threshold to approximately twice the control threshold in about one-third of the animals tested, but was without effect in the other two-thirds, independent of the dosage used (1). The phenomenon of spontaneous defibrillation was not observed with any of the other agents tested.

The Use of Calcium, Norepinephrine, and Bretylium to Antagonize the Myocardial Depression Induced by Antiarrhythmic Agents

Bretylium had both positively inotropic and antifibrillatory actions, while the other agents were negatively inotropic and had either inconsistent antifibrillatory action (propranolol), or no measurable effects on this parameter (quinidine, lidocaine, procaine amide, and diphenylhydantoin). To investigate a possible relationship between inotropic and antifibrillatory actions of the depressant antiarrhythmic drugs, fibrillation thresholds were measured after the depression was antagonized with calcium, norepinephrine, or bretylium.

Myocardial depression induced by propranolol (0.1–0.15 mg/kg) was much more resistant to antagonism by either norepinephrine or bretylium (Fig. 2) than the other agents. Antagonism by bretylium often required the prior administration of norepinephrine.

It was found that calcium was the most potent antagonist to the myocardial depression induced by propranolol. Figure 4 shows a continuation of the same experiment recorded in Figure 2. The heart had been already severely depressed by the administration of propranolol (2 mg) and norepinephrine (12 μg), and bretylium (50 mg) did not augment contractile strength at this time. The administration of 100 mg of calcium gluconate (Fig. 4) caused a marked increase in stroke volume and pressure, as well as a decrease in diastolic volume, antagonizing the myocardial depression induced by propranolol. Although this effect was transient, at its peak the augmented force of contraction actually exceeded the pre-propranolol control level (Fig. 2). Prior to the administration of calcium, norepinephrine (12 μg) had virtually no influence on contractility (Fig. 2). When norepinephrine (10 μg) was given while the positive inotropic action of calcium was declining but still strong (Fig. 4, second arrow), the smaller dose of norepinephrine had a clear positive inotropic action. However, a second bolus of norepinephrine (10 μg) given a few minutes later (when the calcium effect was almost gone) did not alter contractility (third arrow).

Repeated injections of calcium produced the same transient improvement of myocardial contractility, but each time the decline in contractile strength was less, so that after 3 or 4 grams of calcium had been given, the stroke volume and pressure were often restored to the control level and the propranolol induced depression completely antagonized.

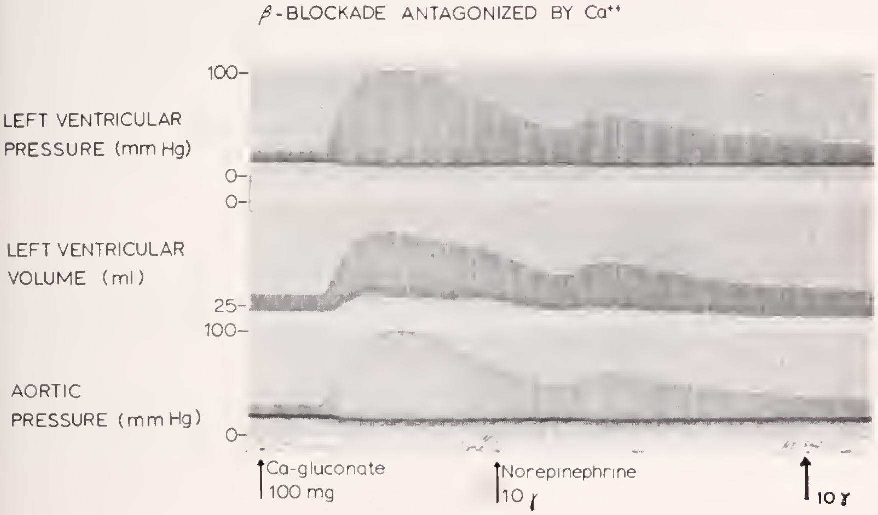

FIG. 4. See text.

The transient nature of the positive inotropic effect of a bolus of calcium or bretylium is due to washout from the heart and dilution in the reservoir (2,000 ml) and tubing. When these agents are continuously infused into the coronary circulation, the positive inotropic effect reaches a plateau and remains elevated.

Figure 5 shows another experiment in which propranolol (1.5 mg) was given to a spontaneously beating isolated heart. In the spontaneously beating heart there was a fall in ventricular pressure and stroke volume, as well as heart rate, without an increase in diastolic volume. Norepinephrine (2 μg) had little effect in antagonizing the myocardial depression or heart rate. The administration of 50, then 100 mg of calcium, caused stepwise proportional increments in contractile strength, and also increased heart rate (second panel). The lower panel shows that the continuous infusion of calcium progressively augments contractile strength, which then reaches a stable plateau. When the calcium infusion was discontinued, contractility declined. The inotropic effect of norepinephrine was restored by the calcium infusion (last two arrows).

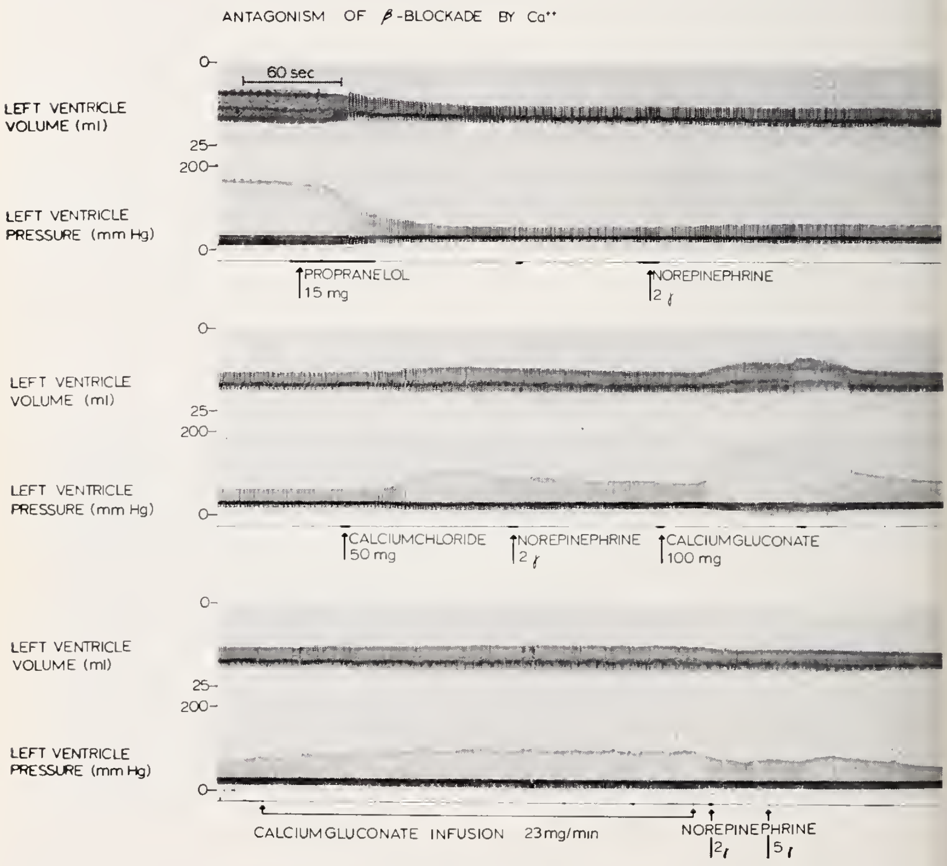

FIG. 5. See text.

Discussion

ANTAGONISM TO MYOCARDIAL DEPRESSION

These experimental studies indicate that the depressant effects of all the agents tested, except propranolol, may be readily antagonized by norepinephrine, calcium, and bretylium (which is itself positively inotropic). Propranolol, however, produces a more severe and refractory depression of myocardial function than the other agents tested. Moreover, cardiac depression induced by propranolol was difficult to antagonize. Even large doses of norepinephrine often failed to return mechanical performance to normal levels. Only the continuous infusion of calcium in large doses produced satisfactory antagonism, and even then it was sometimes necessary to supply norepinephrine concomitantly to restore normal contractility. Strontium (another divalent cation) was also found to antagonize the depression induced by propranolol, but somewhat less effectively than calcium (10). Calcium and digitalis have been reported to antagonize the depressant effect of dichloroisoproterenol, another beta-blocking agent (11).

The interactions of calcium and norepinephrine on the beta adrenergic blocked heart were of considerable interest. Propranolol depressed the dose-response curve (contractile strength) to increasing doses of norepinephrine. However, if the dose-response curves to norepinephrine were repeated while calcium was continuously infused, there was marked shift toward restoration of the normal contractile response (unpublished observations).

The administration of calcium to the beta-blocked heart increased the strength of contraction and the heart rate, and restored the response to low doses of norepinephrine. These findings suggested the possibility that the myocardial depression induced by beta adrenergic blockade may be related to its interference with the normal action of calcium ions in the contractile process. The fact that strontium had a qualitatively similar effect supports this possiblity. The interaction of calcium and norepinephrine in the presence of beta-blockade is difficult to interpret, except to support the idea that facilitation of the action(s) of calcium ions on the contractile mechanism may be an important aspect of the inotropic action of norepinephrine.

MECHANICAL PERFORMANCE AND VULNERABILITY TO VENTRICULAR FIBRILLATION

Our studies in the isolated heart did not reveal any relationship between myocardial contractile state and fibrillation threshold when myocardial depression was antagonized by norepinephrine or calcium.

Bretylium given after any of the other agents usually elevated fibrillation threshold independently of its effect on contractile strength. The propranolol depressed heart often had a large increase in fibrillation threshold after bretylium administration when no positive inotropic effect could be observed.

Bretylium was unique in both its positive inotropic effect and its effect on rate. In the isolated heart beating spontaneously, bretylium had little effect on sinus rate. However, when the His bundle was cut, bretylium increased the idioventricular rate to 2 to 3 times the control rate.

Recent clinical reports on bretylium in the treatment and prevention of arrhythmias have been favorable (12–16). Bretylium blocks postganglionic sympathetic nerve transmission, inducing a state roughly that of a chemical sympathectomy, including hypersensitivity to catecholamines (17), which probably accounts for its positive inotropic effect. In man, bretylium is reported to increase cardiac output by 0.65 l/m^2/minute in both normal and hypertensive subjects (18). The positive inotropic effect of bretylium has been reported in rabbit atria (19) and in intact dog (20).

Because of the general myocardial depressant effect of antiarrhythmic agents, their clinical use must take into account the prior state of myocardial function. When cardiac contractile strength is impaired as in heart failure and shock, or impulse formation is disturbed as in heart block, the use of depressant antiarrhythmic agents may be more deleterious to the patient than the potential benefit obtained from correcting the arrhythmia. The advantage of a positive inotropic action in an antiarrhythmic agent is obvious, and suggests that bretylium may be used clinically when other antiarrhythmic agents would be hazardous.

Summary

In the isolated heart under conditions of constant loading and rate, it was found that quinidine, procaine amide, lidocaine, diphenylhydantoin, and propranolol were negatively inotropic, and that bretylium was positively inotropic. Antagonizing the myocardial depression with norepinephrine or calcium did not alter vulnerability to ventricular fibrillation. Calcium infusion antagonized propranolol induced myocardial depression and restored the positive inotropic effect of norepinephrine.

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Modification of the Systolic Ejection Gradient of Ventricular Outflow Obstruction by Paired Electrical Stimulation and Changes in Downstream Resistance

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Paired electrical stimulation of the heart has been demonstrated to produce striking alterations in the performance of the ventricle. Its powerful inotropic influence results in a marked enhancement in the force of myocardial contraction, with significant increase in stroke power, speed of ventricular contraction, rate of intraventricular pressure rise, and velocity of blood flow in the aorta (1). Improved ventricular emptying has been demonstrated in heart failure with reduction in end-diastolic pressures (2). In addition, since the technique of paired pacing doubles the mechanical refractory period of the ventricle, the heart rate is effectively slowed. The method of coupled pacing allows the normal, spontaneous ventricular depolarization to act as the initial beat of the pair, and has the advantage of retaining the atrial contribution to ventricular filling, as well as allowing the depolarization wave to be conducted over the normal pathway. The improved ventricular performance during coupled pacing results from the combined effects of augmentation due to increased filling that occurs during the longer diastolic period, and inotropic potentiation by the premature beat. In coupled pacing the ventricular rate is exactly halved, and it is this method that is largely used in the present study.

Conflicting statements have been made concerning the effect of sustained post-extrasystolic potentiation on cardiac output and myocardial oxygen consumption (3, 5, 8). There is no question that paired electrical stimulation strikingly increases the myocardial oxygen requirement, and that minute cardiac output generally does not increase in the non-failing preparation, if the heart rate is held constant (1). However, if the heart rate is slowed, the stroke output is augmented to maintain minute output, and the oxygen requirement for the reduced number of ventricular contractions may not increase above normal (4).

Sustained post-extrasystolic potentiation has been demonstrated to improve the performance of the failing ventricle by a simultaneous increase in cardiac output and reduction in left ventricular end-diastolic pressure (2, 5). It is the purpose of this paper to illustrate the effect of paired and coupled electrical stimulation on the hemodynamics of a ventricular stress situation other than heart failure, namely experimental aortic or pulmonic stenosis.

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Methods

A left thoracotomy was performed on adult mongrel dogs, anaesthetized with intravenous sodium nembutal, and supported with positive pressure respiration through an endotracheal tube. The base of the aorta was dissected free and a linen noose passed about the supravalvular aorta or pulmonary artery. Aortic blood flow was measured with a Medicon electromagnetic flow meter.

The flow-probe was applied about the aorta just distal to the supravalvular constriction. An intracardiac phono-catheter was inserted into the ascending aorta through the carotid artery to record pressure pulses and heart murmurs immediately downstream to the area of stenosis. A bipolar electrode catheter was passed into the left atrium and ventricle via a branch of a pulmonary vein. Pressures, sounds, stroke output, dp/dt, or df/dt were recorded on an eight channel Electronics for Medicine oscillograph. The heart was paced with a Medtronic R-wave Coupled Pulse Generator, either utilizing paired stimulation at varying heart rates, or coupled pacing to slow the rate by 50 percent. Peripheral arterial resistance was elevated by acutely compressing the aorta at the level of the diaphragm. Pulmonary vascular resistance was raised by sudden increases in airway resistance produced by occluding the expiratory vent of the respirator.

Results

CHANGES IN EJECTION GRADIENT PRODUCED BY PAIRED OR COUPLED PACING

Figure 1 illustrates an example of coupled electrical stimulation of the heart of a dog with aortic stenosis. Peak systolic ejection gradient rose from 30 mm

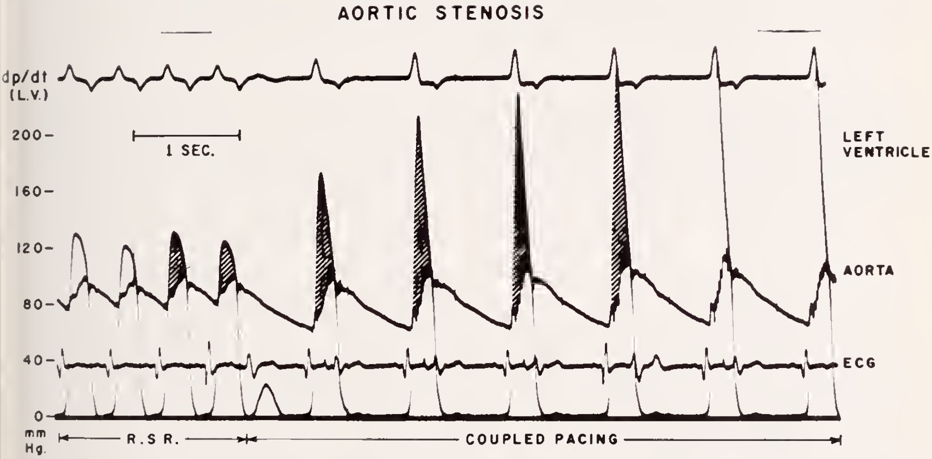

FIG. 1. The pressure gradient and rate of rise of left ventricular pressure during coupled pacing in aortic stenosis. The systolic ejection gradient quadrupled and the maximum velocity of left ventricular contraction (dp/dt, maximum) increased 150 percent. Left ventricular end-diastolic pressure was unchanged.

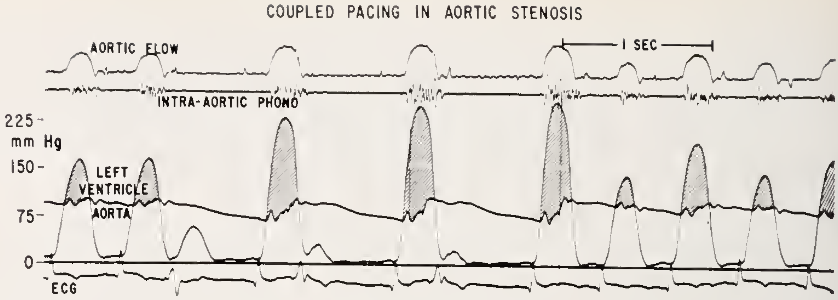

FIG. 2. Response of pressure pulses, stroke output, and systolic murmur to coupled pacing in aortic stenosis. The systolic ejection gradient rose from 70 mm Hg to 165 mm Hg (130 percent) while the stroke output showed an increase of 85 percent. The heart rate was halved. Systolic ejection time rose 15 percent and the systolic murmur became louder and longer. Following the cessation of pacing, ventricular alternans occurred.

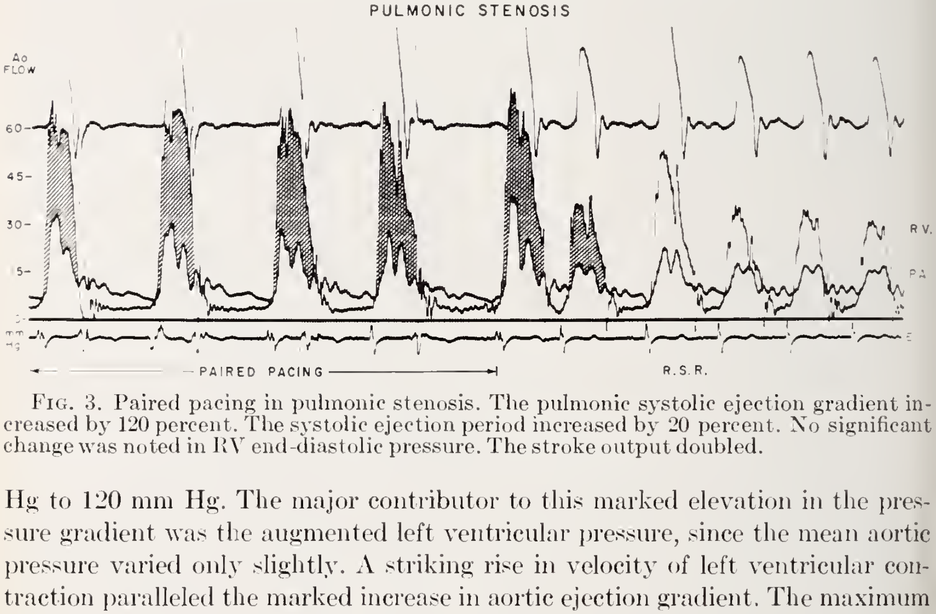

FIG. 3. Paired pacing in pulmonic stenosis. The pulmonic systolic ejection gradient increased by 120 percent. The systolic ejection period increased by 20 percent. No significant change was noted in RV end-diastolic pressure. The stroke output doubled.

Hg to 120 mm Hg. The major contributor to this marked elevation in the pressure gradient was the augmented left ventricular pressure, since the mean aortic pressure varied only slightly. A striking rise in velocity of left ventricular contraction paralleled the marked increase in aortic ejection gradient. The maximum rate of rise of the left ventricular pressure (dp/dt) increased by 150 percent. As shown in Figure 2, stroke aortic flow increased by 85 percent during coupled pacing, but minute output was essentially unchanged since the heart rate was halved. Despite the marked increase in stroke output and ventricular peak systolic pressure, there was no significant change in left ventricular end-diastolic pressure. In a group of dogs paced at various heart rates, minute cardiac output, left ventricular end-diastolic pressure, and mean aortic pressure showed no significant change during paired pacing. Similar effects were noted in pulmonic stenosis (Fig. 3).

Following the cessation of pacing, the ejection gradient remained elevated during the gradual post-drive decay of ventricular pressure (Fig. 4). This indicated that a significant part of the rise in ejection gradient was due to the positive

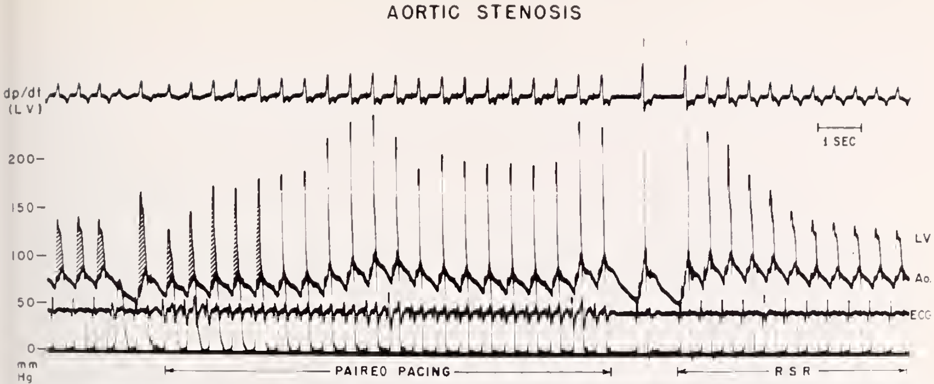

FIG. 4. Paired pacing in aortic stenosis. The magnification of the ejection gradient and increase in velocity of left ventricular pressure rise are demonstrated. Note the gradual decay of LV pressure and ejection gradient following the cessation of pacing.

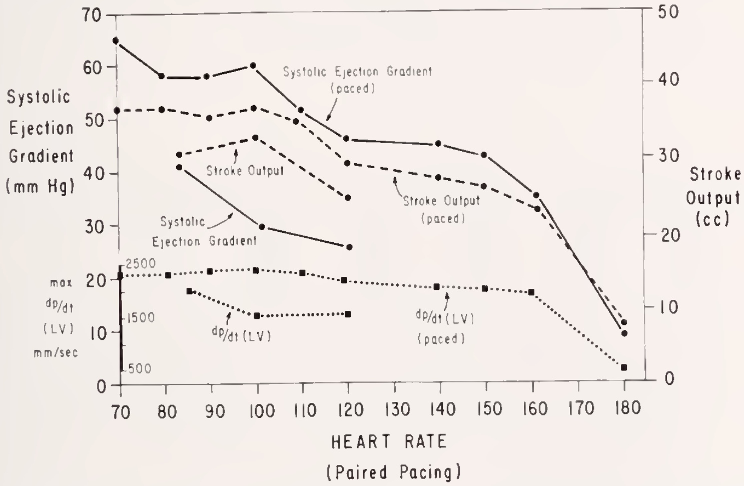

FIG. 5. Relation between systolic ejection gradient, aortic stroke output, and dp/dt (LV) at varying paired pacing rates, compared with non-paced controls (short lines). The gradient and stroke outputs are maximal up to rates of 100/minute and are markedly depressed when paced rates exceed 160/minute. Paced and control outputs are similar, but paced ejection gradients are significantly greater than control gradients, up to rates of 160/minute. Velocity of left ventricular contraction (dp/dt) remains elevated until the pacing rate becomes excessively rapid.

inotropic influence of paired electrical stimulation, and that the prolonged diastolic filling period during pacing was only partially responsible for the increase in gradient. During paired pacing at rates closely approximating the control rate, the rise in pressure gradient was less marked. If pacing rates were excessively fast, stroke volume, aortic ejection gradients, and peak left ventricular systolic pressures were distinctly depressed (Fig. 5).

VENTRICULAR ALTERNANS DURING AORTIC STENOSIS

The ventricular alternans that occurred when the left ventricle was stressed by aortic constriction, or which followed paired pacing, took several forms:

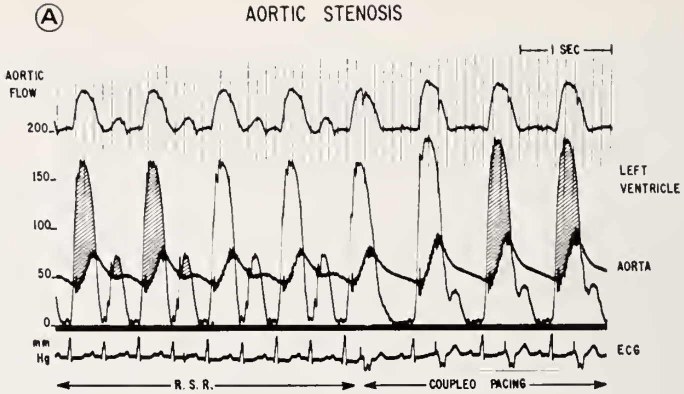

Fig. 6A. Ventricular alternans in aortic stenosis. The first four couplets represent spontaneous ventricular alternans, with weaker beats just effective enough to open the aortic valve. The last three beats demonstrate the effect of coupled pacing, which is accompanied by an increase of 20 percent in stroke output.

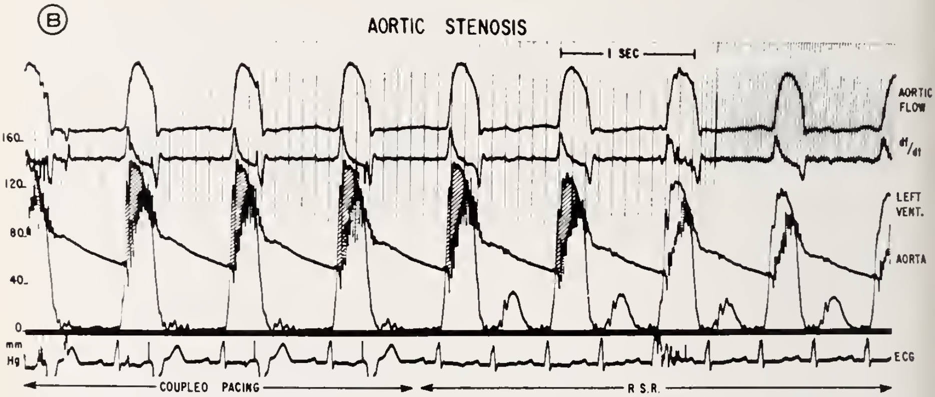

Fig. 6B. Ventricular alternans in aortic stenosis following cessation of coupled pacing. Aortic flow is nonexistent when the frustrate smaller beats fail to eject blood, as illustrated in the aortic flow and rate of flow (df/dt) traces.

a complete form of alternans (Fig. 6B) in which the smaller beat of the pair was completely frustrate and yielded no aortic flow; an incomplete form (Fig. 6A) where the aortic valve opened, but stroke output was distinctly less than the normal; and a combination of electrical and mechanical alternans (Fig. 6A). Each of these was eliminated by coupled pacing. When alternans was present, a further tightening of the stenosis led to the appearance of bigeminal rhythm (Fig. 7). Bigeminal rhythm simulated complete ventricular alternans mechanically, but alternate beats arose from a clearly ectopic focus. During the various forms of alternans, the left ventricular stroke output varied directly with the velocity of contraction, peak left ventricular pressure, and aortic ejection gradient. The systolic ejection phase of the stronger beat was prolonged and encroached upon the diastolic filling period of the succeeding weaker beat. There was no constant relation between end-diastolic pressure and the magnitude of

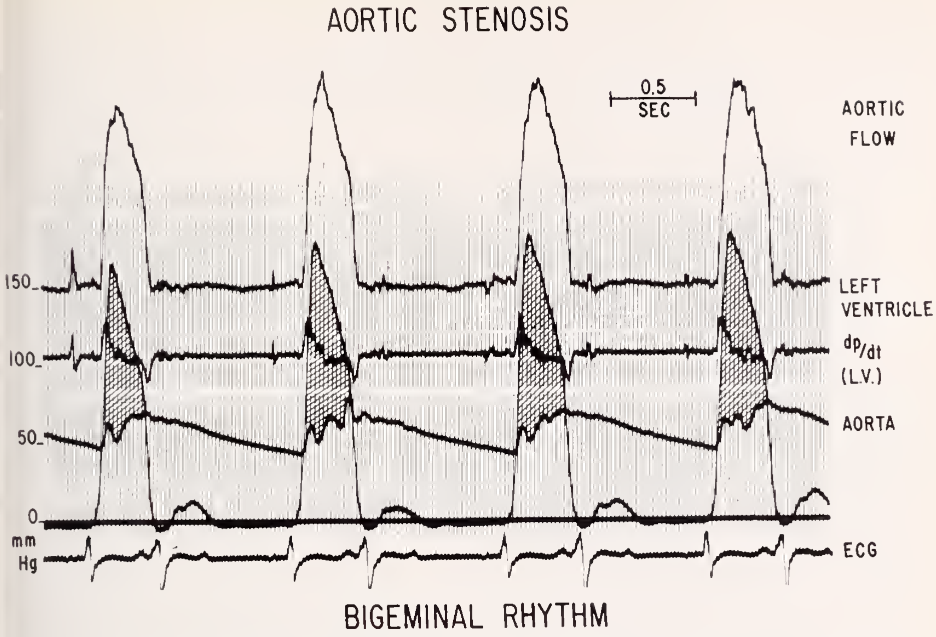

FIG. 7. Spontaneous bigeminal rhythm which replaced ventricular alternans when the supravalvular aortic stenosis was increased. The resemblance to the hemodynamic effects of coupled pacing is obvious.

the succeeding beat. The weaker beat demonstrated a decrease in stroke output and velocity of contraction, and there was less left ventricular stroke work loss than in the more powerful beats. When bigeminal rhythm replaced ventricular alternans, this change in mechanism was accompanied by a significant increase in stroke output, closely simulating the response to coupled pacing. Not only did the intensity of the systolic murmur correlate with the peak pressure level and stroke output of the alternating beats, but the delay between first heart sound and the onset of the systolic mumur reflected the diminished velocity of isovolumic contraction of the weaker beat. In addition, the shorter duration of the systolic murmur produced by the weaker beat confirmed the reduction in systolic ejection time of the smaller beat (Fig. 2).

MODIFICATION OF THE EJECTION GRADIENT IN AORTIC AND PULMONIC STENOSIS BY CHANGES IN DOWNSTREAM RESISTANCE

Figure 8 demonstrates the effect of an acute increase of the peripheral vascular resistance in a dog with aortic stenosis. Following constriction of the descending aorta, the ejection gradient was sharply reduced, primarily due to an abrupt rise in aortic mean pressure. This was accompanied by a small reduction in stroke output. The hemodynamic alterations abruptly disappeared with release of the downstream constriction. With reduction in the aortic ejection gradient,

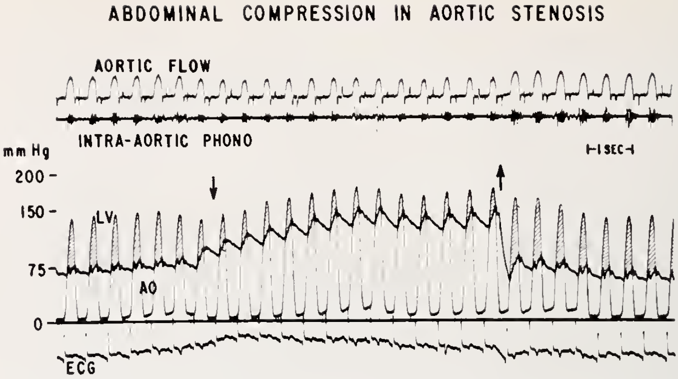

Fig. 8. Response of the ejection gradient in aortic stenosis to a second source of downstream resistance. When the abdominal aorta is compressed, the ejection gradient is reduced by 40 percent, primarily due to a rise in aortic mean pressure. There is a 30 percent reduction in stroke output during compression, and the systolic ejection murmur becomes shorter and less intense.

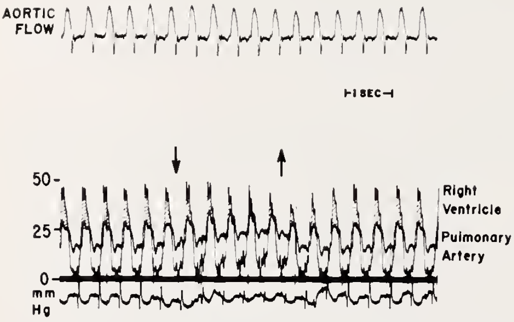

Fig. 9. Reduction in ejection gradient in supravalvular pulmonic stenosis when expiratory airway resistance of respirator is acutely increased (first arrow). The narrowed gradient is due primarily to a rise in pulmonary artery pressure. Right ventricular systolic pressure falls and end-diastolic pressure rises when the obstruction to outflow is prolonged. Aortic flow decreased 15 percent.

there was an accompanying diminution in the intensity and duration of the ejection murmur.

The response of the right side of the heart to an increase in pulmonary vascular resistance is shown in Figure 9. As the downstream resistance rises, due to an acute elevation of airway resistance, the gradient of supravalvular pulmonic stenosis diminishes. Elevation of right ventricular end-diastolic pressure takes place if the downstream obstruction is sustained.

Discussion

The severity of aortic and pulmonic valve stenosis has been related primarily to the rate of forward flow and the ejection gradient. Little attention has been paid to changes in gradient which can be produced by alterations in myocardial contractility and peripheral vascular resistance, often independent of changes in output.

The striking elevation of ejection gradient demonstrated during coupled pacing indicates that this positive inotropic influence plus prolonged diastolic filling combine to augment the gradient across a fixed stenosis. Enhanced ventricular contractility results in increased velocity of blood flowing through the stenotic orifice. With high volume flow across a narrowed aortic valve there is a marked loss of kinetic energy, and ventricular pressure decays rapidly in conversion to velocity. Gorlin and Gorlin (6) pointed out that changes in velocity of flow through stenosed valves are proportional to the pressure gradient, but if velocity of flow across a fixed stenosis increases during paired pulse stimulation at the control rate, without an accompanying increase in stroke output, the resultant magnification of the ejection gradient is not accounted for by the Gorlin formula. Errors can occur in clinical estimation of orifice area if the effects of inotropic influences on contractility are ignored, or if minute cardiac output is considered in relation to the ejection gradient, rather than stroke output.

On the other hand, for a fixed degree of stenosis, the ejection gradient may be clinically underestimated if there is a second source of downstream resistance distal to the stenosis. It has been amply demonstrated by the studies of Silove, Vogel, and Grover (7), and confirmed by the experiments in this report, that modification of the peripheral vascular resistance may cause marked fluctuation in the aortic or pulmonic ejection gradient. Increases in peripheral arterial resistance downstream to the site of ventricular outflow obstruction may reduce or even obliterate a pressure gradient at, or near, the valve. Clinical examples related to this phenomenon are the unmasking of infundibular stenosis that may follow pulmonary valvotomy (9), or the elimination of the systolic pressure gradient in hypertrophic obstructive cardiomyopathy by pharmacologic elevation of peripheral arterial resistance (10). Silove et al (7) have pointed out that this phenomenon is not limited to dynamic forms of muscular outflow obstruction and may be demonstrated across the fixed mechanical obstruction of discrete aortic valvular stenosis. This change in ejection gradient by modifying peripheral vascular resistance can at times be induced without a concomitant change in orifice flow.

It is of interest to compare the two mechanisms of altering the pressure gradient across a fixed aortic stenosis. When an acute increase in resistance is produced by distal aortic compression, it is mean aortic pressure that rises to a significantly greater extent than peak ventricular pressure, thereby reducing the gradient. Contrariwise, when contractility of the ventricle is enhanced by paired pulse stimulation, the aortic mean pressure remains virtually constant while left ventricular peak pressure rises to a greater extent, magnifying the ejection gradient. The positive inotropic effect of paired pacing has been shown to exceed that of an acute increase in afterload (11).

It has long been recognized that ventricular alternans can occur whenever the ventricle is placed under stress. A great deal of experimental and clinical investigation has been undertaken to explain the mechanism of the mechanical alternans seen in aortic stenosis (12–16). There are two current theories. First, the Starling mechanism states that alternation in diastolic volume leads to alternation in contractile force. The second hypothesis is the myocardial contractility theory, which holds that there is an alternation of the basic inotropic state of the entire myocardium, or in the number of cardiac fibres contributing to each contraction, without changes in end-diastolic volume or fibre length. Alternation in end-diastolic volume may actually be the consequence rather than the cause of the alternating pulse, since the stronger beat presents a smaller end-diastolic volume to the succeeding beat and occupies a greater portion of the diastolic filling period. Much of the hemodynamic data has been based on indirect methods of detection of changes in stroke volume, such as angiographic determinations (14, 17). These studies have shown that the smaller beat has a decreased stroke volume, reduced duration of ejection, reduced peak flow, prolonged isometric contraction phase, and a reduction in the rate of ventricular pressure development. The actual beat-to-beat stroke volume measurements in the present study would tend to confirm these findings.

There appears to be a continuum of hemodynamic changes in ventricular alternans, bigeminal rhythm, and coupled electrical stimulation which suggests a modus operandi for the alternans phenomenon. It is an old clinical observation that some patients with cardiomegaly, congestive heart failure, and low output syndrome may spontaneously improve when they develop bigeminal rhythm (2). In our experiments, when ventricular alternans followed the production of aortic stenosis, a slight additional increase in the stenosis resulted in the alternans being replaced by bigeminal rhythm. This was accompanied by a significant increase in stroke output, simulating the response to coupled pacing. It is an attractive hypothesis to postulate that the ventricle exposed to progressively increasing afterload was attempting to initiate coupled pacing. This observation suggested that ventricular alternans may be a feeble attempt, utilized early in a sequence of mechanisms available to the stressed ventricle, to invoke the inotropic benefits of sustained post-extrasystolic potentiation.

Summary

Manipulation of the ejection gradient in fixed ventricular outflow obstruction in dogs was accomplished by paired electrical stimulation and by increasing the downstream resistance distal to the stenosis. The positive inotropic influence of paired or coupled pacing caused increases in the aortic ejection gradient up to 300 percent of the control. The greatest increases were seen as the heart rate was slowed, when the effects of enhanced diastolic filling and post-extrasystolic potentiation combined to magnify the gradient. However, even when pacing and control rates and stroke outputs were similar, the gradient rose significantly with paired pulse stimulation. An acute increase in downstream resistance, distal to the stenosis, caused either by descending aortic occlusion or increased airway resistance, significantly reduced (20–60 percent) the aortic or pulmonic ejection gradient. Ventricular alternans often followed the cessation of paired pacing and was encountered whenever ventricular afterload was increased. Coupled pacing duplicated the hemodynamic effects of the spontaneous bigeminal rhythm that superseded ventricular alternans when the outflow obstruction was intensified.

Acknowledgment

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Analysis of Atrioventricular Conduction by Direct Intracardiac Recordings*

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Introduction

In a recent editorial, Cranefield and Hoffman (1) pointed out some of the limitations of the surface electrocardiogram in the analysis of atrioventricular conduction disturbances. Specifically, there are no deflections in the peripheral electrocardiogram relating to the spread of atrial activity through the atrioventricular node, the His bundle, and the bundle branches. These events for the most part occur during the isoelectric period of the P-R interval.

A recent report describes a technique for recording from the His bundle in the human heart during right heart catheterization (2). Using this technique, preliminary studies from this laboratory have localized conduction defects to the proximal and distal atrioventricular junction.

The purpose of this report is to present recordings of His bundle electrograms in patients with normal A-V conduction or various types of conduction defects. In addition, we have extended the electrode catheter recording technique to the right and left bundle branches in man and the dog heart, respectively. A review of the development of the His bundle recording technique in man, its validity and possible clinical significance are presented.

Methods and Definitions

RECORDING OF HIS BUNDLE ACTIVITY

One hundred patients were studied in the post-absorptive state during right heart catheterization. All patients were premedicated with 100 mg Nembutal® IM 1 hour prior to the study. Most patients had underlying cardiac disease; however, from an electrical standpoint these patients either had normal A-V conduction or some degree of A-V block. A 5 Fr standard pacing catheter with ring electrodes (U.S. Catheter Co., Glen Falls, N.Y.), or a specially constructed multipolar catheter (Elecath Corp., Rahway, N.J.) was introduced percutaneously into the right femoral vein using a modified Seldinger technique. The catheter was advanced into the right ventricle under fluoroscopy and with electrographic monitoring. By slowly withdrawing the catheter to the tricuspid valve area, bipolar recordings of His bundle activity were obtained (2). A unipolar electrogram from the same electrode catheter, as well as standard electrocardiographic leads I, AVR, and AVF, or 1, 2, and 3, were

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* Supported in part by Florida Heart Association, Grant No. 69AG11.

monitored and registered using an Electronics for Medicine oscillographic, photographic multichannel recorder.

Another bipolar electrode catheter was introduced into the right atrium through an antecubital vein. The catheter tip was positioned against the lateral atrial wall for atrial pacing and the electrical induction of premature atrial stimuli. A Medtronic battery powered pacemaker (Model 5784) was used in the fixed rate pacing mode for atrial pacing from a rate 10 beats higher than the sinus rate to rates of 150 or 180 per minute or until incomplete A-V block developed. In addition, with the pacer in the synchronous mode, a single premature atrial stimulus could be delivered to the atrium with a variable interval (10–300 m sec) from a preceding QRS complex. All equipment was carefully grounded to eliminate stray AC currents from entering the patient-recorder circuit. There were two groups of patients studied: those with normal A-V conduction (Group I), and those with A-V conduction defects (Group II). Subgroups of patients were studied within the general Group II category of A-V conduction defects: (1) first degree A-V block; (2) second degree A-V block; (3) right bundle branch block with left axis deviation; and (4) complete heart block.

RECORDING OF ELECTRICAL ACTIVITY FROM THE RIGHT BUNDLE IN MAN AND THE LEFT BUNDLE IN THE DOG

The introduction or withdrawal of the bipolar electrode catheter from the right ventricle in man usually permitted recording of activity from the right

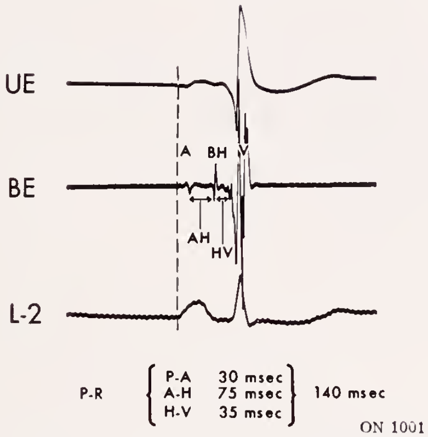

Fig. 1. Simultaneous recording of unipolar and bipolar electrograms from the area of the A-V junction and lead 2 ECG. The components of the P-R interval are: (1) intra-atrial conduction time (P-A); (2) A-V nodal conduction time (A-H); and (3) His-Purkinje system conduction time (H-V). P-A time = the time interval from the onset of the P wave to the A wave; A-H time = the time interval from the A wave to the Bundle of His (BH) deflection; H-V time = the time interval from BH to the onset of ventricular activation (V). A = bipolar atrial electrogram from the area of the A-V junction; BH = bipolar electrogram of the Bundle of His; V = bipolar ventricular electrogram from the area of the A-V junction; UE = unipolar electrogram from the area of A-V junction; BE = bipolar electrogram from the area of A-V junction; L-2 = lead II, a standard lead II electrocardiogram.

TABLE I

Measurements of Time Intervals between Intracardiac Electrical Events Occurring during the P-R Interval in Man

Patient No.	Conduction Times (m sec)					
	Basal HR	P-R	P-A	A-H	H-V	Diagnosis
1	75	170	50	80	40	Left axis deviation
2	62	230	60	120	50	1° HB, normal axis
3	80	200	30	120	50	Sinus bradycardia, MI anterior wall
4	65	200	50	110	40	Horizontal axis, nonspecific ST-T wave
5	75	150	50	65	35	Left axis, nonspecific ST-T wave
6	68	200	45	120	35	Nonspecific ST-T wave
7	50	190	55	95	40	Normal axis, sinus bradycardia
8	57	210	45	130	35	Sinus bradycardia
9	50	160	30	90	40	Normal axis, sinus bradycardia
10	63	140	25	80	35	Normal heart
11	86	160	50	70	40	Mild obstructive lung disease
12	63	170	40	90	40	Mild obstructive lung disease
13	73	160	60	60	40	Mild obstructive lung disease
14	55	200	65	95	40	ASHD ?
15	57	170	35	95	40	ASHD
16	60	160	60	60	40	Systolic hypertension
17	71	140	45	50	45	Small ASD
18	80	150	40	70	40	ASHD
19	55	190	30	120	40	PDA
20	63	200	50	105	45	ASHD, hypertension
21	62	160	30	90	40	ASHD, hypertension
22	77	160	25	90	45	Chronic obstructive lung disease
Average	66	176	44	91	41	
Standard deviation	±10	±24	±14	±23	±4	

bundle branch and/or the vicinity of the junction of the right bundle branch and the anterior papillary muscle. The identification of these recordings was performed on the basis of previous description of such activity in animals in which electrodes were placed in direct contact with the right bundle branch during cardiotomy and cardiopulmonary bypass (3, 4).

In four anesthetized dogs (Na-pentobarbital, 30 mg/kg) placed in the supine position, electrode catheters as described above were introduced into the right heart via a femoral vein to record His bundle activity (5, 6). Catheters were also passed from the left common carotid artery or femoral artery into the left ventricle to record from the main left bundle and its posterior terminus. These catheters were positioned only by electrocardiographic monitoring, and when the experiments were terminated the catheter positions were verified by direct visualization of the opened hearts.

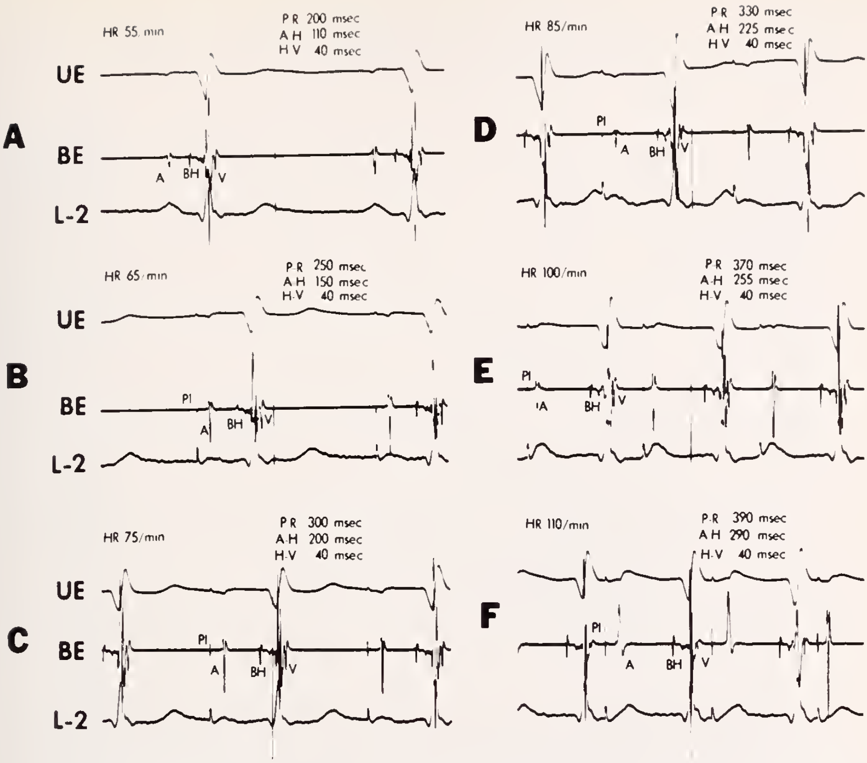
A
HR 55/min
P-R 200 msec
A-H 110 msec
H-V 40 msec
UE
BE
L-2
A
BH
V
B
HR 65/min
P-R 250 msec
A-H 150 msec
H-V 40 msec
PI
C
HR 75/min
P-R 300 msec
A-H 200 msec
H-V 40 msec
D
HR 85/min
P-R 330 msec
A-H 225 msec
H-V 40 msec
E
HR 100/min
P-R 370 msec
A-H 255 msec
H-V 40 msec
F
HR 110/min
P-R 390 msec
A-H 290 msec
H-V 40 msec

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FIG. 2. Simultaneous unipolar, bipolar recordings from the area of the A-V junction and L-2. A. Normal sinus rhythm with a heart rate of 55/minute. P-R interval = 200 m sec.; A-H time = 110 m sec; and the H-V time = 40 m sec; B., C., D., E., and F. The atrium is paced at progressively increasing rates of 65, 75, 85, 100, and 110/minute, respectively. There is progressive prolongation of the A-H time from 150 m sec, at a rate of 65/minute to 290 m sec, at a rate of 110/minute. At all heart rates the H-V time remained constant at 40 m sec. UE = unipolar electrogram from the area of A-V junction; BE = bipolar electrogram from the area of A-V junction; L-2 = standard lead electrocardiogram; A = atrial electrogram from the area of the A-V junction; BH = Bundle of His electrogram; V = ventricular electrogram from the area of A-V junction; PI = pacer impulse. The interval between time lines is 1 second (1000 m sec).

DEFINITION OF TERMS

The following intervals were measured from the recorded His bundle electrograms and standard ECG leads (see Fig. 1):

(1) P-A = Interval from the onset of the P wave in the surface electrocardiogram, or from the right atrial pacing impulse (PI) during atrial pacing, to the beginning of the A wave, representing local right atrial activity in the unipolar and bipolar recordings.

(2) A-H = Interval from the onset of the A wave to the initial deflection of the His bundle activity in the bipolar recording.

(3) H-V = Interval from the His bundle electrogram to the earliest ventricular activity in the bipolar, unipolar electrogram or any of three different standard surface electrocardiograms (usually leads 1, 2, and 3 were recorded).

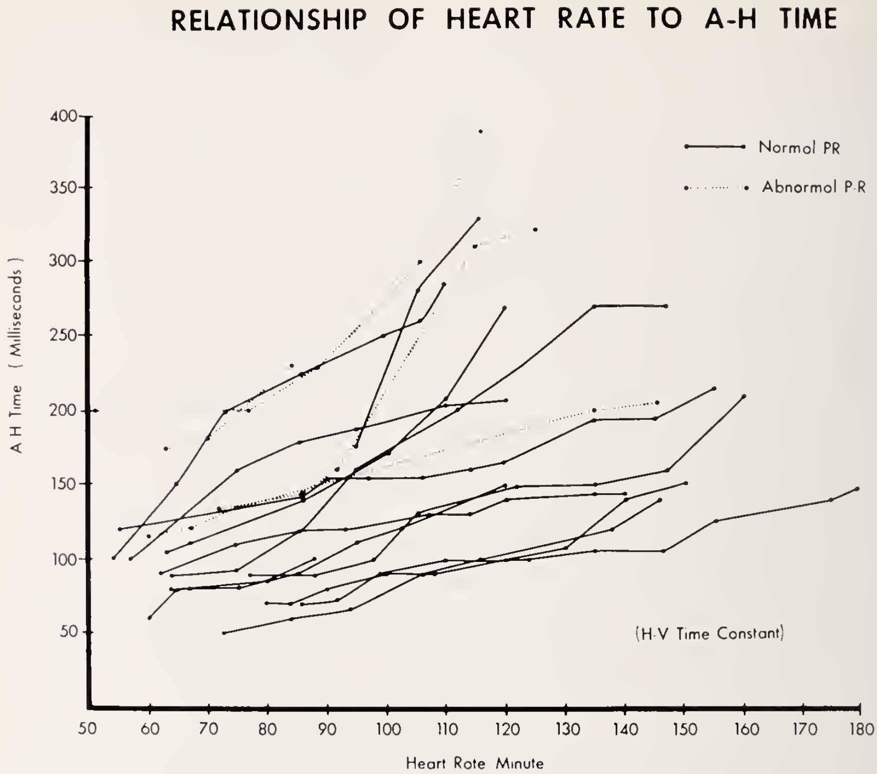

FIG. 3. A graphic representation of the effect of heart rate on A-H time in patients with normal or abnormal P-R intervals. When the heart rate was increased from 50–180 beats/minute by atrial pacing, there was a progressive prolongation of the A-H time, while the H-V time remained constant. It should be noted that the patients with normal P-R interval and the patients with prolonged P-R interval responded qualitatively in the same manner.

(4) P-P = Interval from the beginning of the A wave to the next A wave of any two successive cardiac cycles.

Unipolar electrogram is an intracardiac lead consisting of any one of the two recording sites on the electrode catheter in relation to ground (right leg). This lead is fed directly into a DC amplifier and displayed with low and high frequency limits of 0.1–20 Hz, respectively.

Bipolar electrogram is an intracardiac lead recording the potential difference between the two close recording sites on the electrode catheter. This lead is fed directly into a DC amplified and displayed with low and high frequency limits of 40–200 Hz, respectively.

His bundle electrogram is a bipolar recording from the atrial septal area just above the posterior leaflet of the tricuspid valve. The typical recording shows an electrical signal associated with atrial activity (A wave) which is coincident with some portion of the P wave and the His bundle activity, a rapid biphasic or triphasic spike occurring during the isoelectric period of the P-R interval. Electrical activity associated with ventricular depolarization is

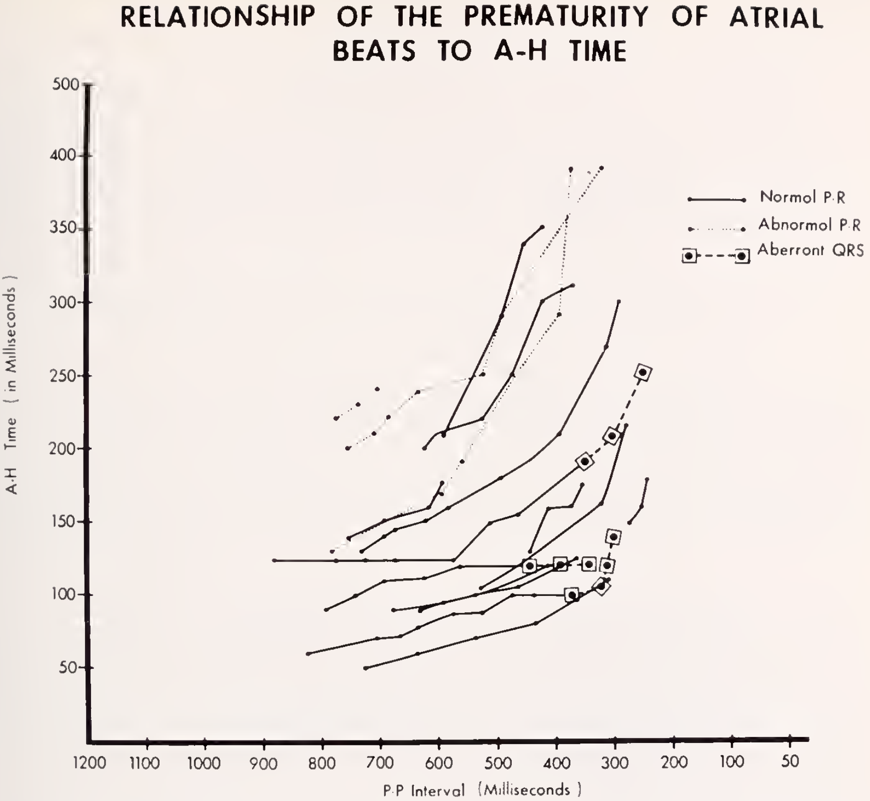

FIG. 4. The relationship of the prematurity of atrial beats to A-H time. As the P-P interval (abscissa) was progressively shortened by the placement of increasingly more premature atrial beats, the A-H time (ordinate) progressively prolonged in all cases. The H-V time remained constant at all heart rates in 13 of 17 cases. In 4 cases the H-V time increased; in each instance the prolongation of the H-V time was associated with aberrant ventricular activation.

also seen in this recording, which coincides with the QRS complex of the standard ECG.

Proximal A-V junction is that part of the specialized conduction system including the A-V node and the junction of the A-V node and His bundle.

Distal A-V junction is that part of the specialized conduction system including the His bundle and the right and left bundle branches and their ramifications.

When the A wave is followed by a His bundle (BH) deflection but not by a ventricular (V) deflection, the site of the block is probably distal to the His bundle, but is also possibly within the distal portion of the His bundle. Conversely, when the A complex is not followed by the BH deflection, the site of block is probably proximal to the His bundle, but is also possibly in the proximal portion of the His bundle. For the sake of simplicity, the former site of block will be described in this study as distal to the His bundle, or distal to the

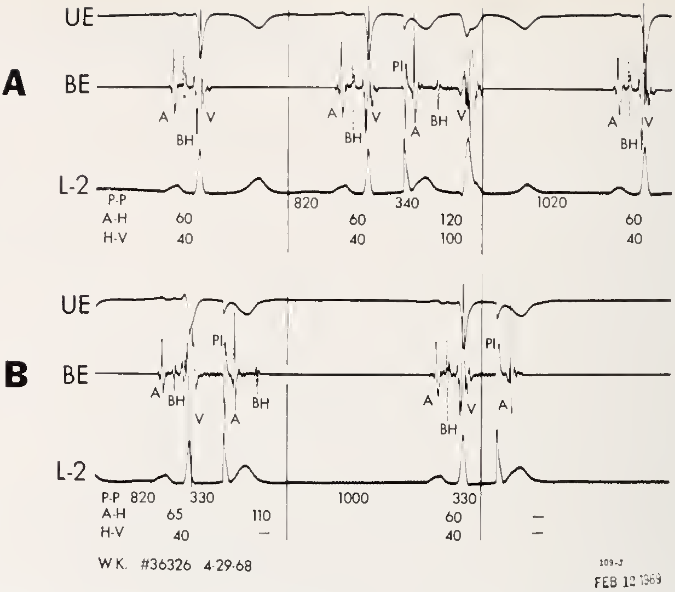
A
UE
BE
L-2
A
BH
V
PI
A
BH
V
P-P
A-H
H-V
60
40
820
60
40
340
120
100
1020
60
40
B
UE
BE
L-2
PI
A
BH
V
A
BH
P-P 820
330
A-H
65
110
H-V
40
—
1000
330
60
40
—
—
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Fig. 5. The effect of premature atrial stimuli on A-H and H-V conduction time. A. The control P-P interval was 820 m sec, and the placement of a premature atrial beat (third beat) at P-P interval of 340 m sec lengthened the A-H time from 60 to 120 m sec. Additionally, there was prolongation of H-V time from 40 to 100 m sec, with aberration of the QRS complex; B. In the first half of this strip when the P-P interval was shortened to 330 m sec, the premature atrial beat was blocked distal to the Bundle of His deflection. A second premature atrial beat following the next QRS at the same P-P interval (330 m sec) produced block proximal to the His bundle deflection.

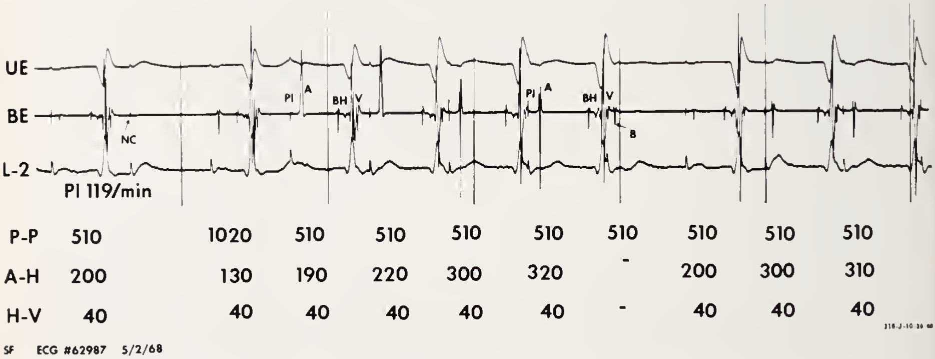
UE
BE
L-2
NC
PI
A
BH
V
PI
A
BH
V
B
PI 119/min
P-P 510 1020 510 510 510 510 510 510 510 510
A-H 200 130 190 220 300 320 - 200 300 310
H-V 40 40 40 40 40 40 - 40 40 40
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Fig. 6. Wenckebach phenomenon during atrial pacing. Atrial pacing at 110/minute. The Wenckebach cycle starts with an A-H time of 130 m sec and P-P interval of 1020 m sec after a pacer impulse that fails to capture the atrium (NC). The P-P interval during the remainder of the record remains constant at 510 m sec, with a progressive prolongation of the A-H time to 320 m sec, culminating in a blocked A wave (B) in the proximal A-V junction. Note that the A-H time for the beat following the blocked A wave (B) is 200 m sec, as compared to 130 m sec for the beat following the nonconducted beat (NC), representing antegrade concealed conduction. The H-V time remains constant at 40 m sec for all the conducted beats.

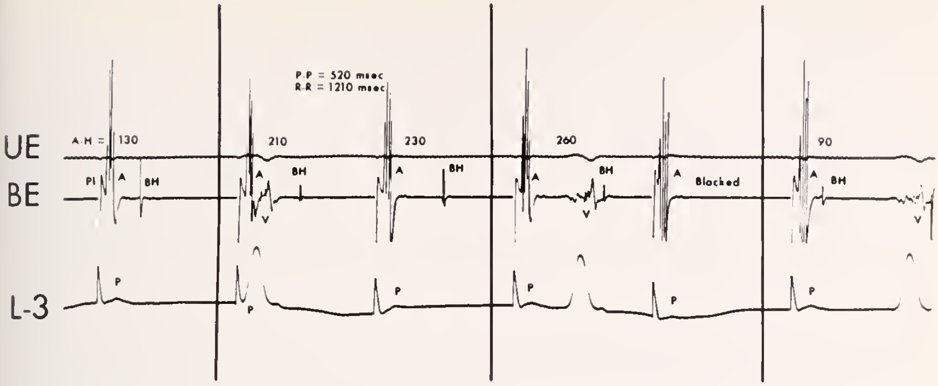
P-P = 520 msec
R-R = 1210 msec
UE
BE
L-3
A-H = 130
210
230
260
90
PI
A
BH
V
P
Blocked

Fig. 7. Atrial pacing induced Wenckebach during complete heart block. In this patient the recording of a unipolar (UE), bipolar (BE), His bundle electrogram and standard lead III (L-3) shows complete A-V block with a constant idioventricular rhythm (V). Note that the block is distal to the His bundle recording. On atrial pacing (PI = pacer impulse) at a rate of 115/minute, Wenckebach cycles occur. The A-H time widens from 130 to 210, 230, and 260 before a blocked beat occurs. The next A-H interval after the blocked A wave is 90 m sec, indicating concealed conduction of the blocked beat preceding the first beat in this series. The ventricular rate is 50/minute.

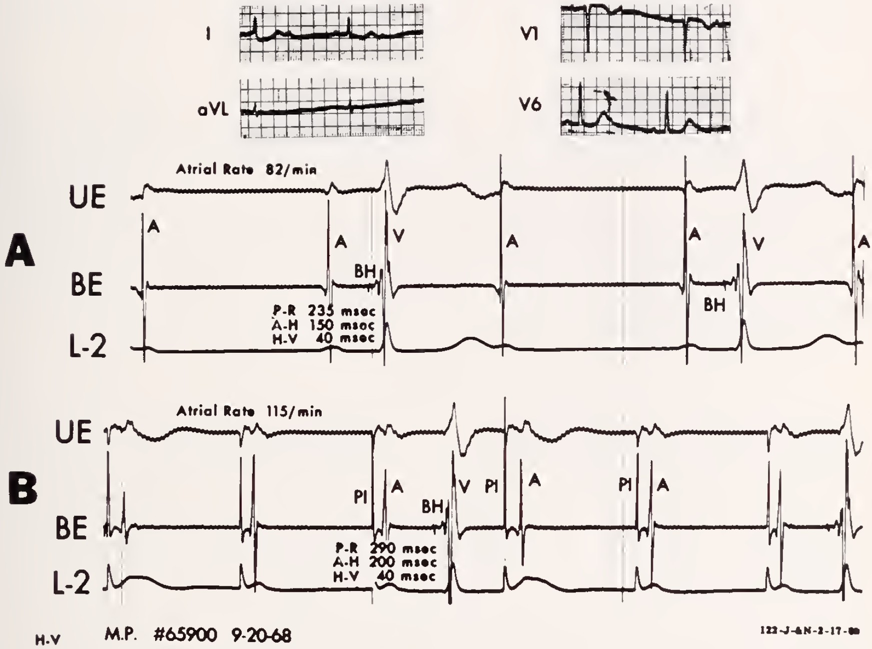
I
aVL
V1
V6
Atrial Rate 82/min
A
UE
BE
L-2
A
V
BH
P-R 235 msec
A-H 150 msec
H-V 40 msec
Atrial Rate 115/min
B
PI
P-R 290 msec
A-H 200 msec
H-V 40 msec
H-V
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Fig. 8. A-H and H-V time during 2:1 and 3:1 A-V conduction. At the top, the standard ECG leads 1, AVL, V1, and V6 show 2:1 A-V conduction with a normal QRS complex. A. The non-conducted A wave (P wave) is blocked proximal to the BH deflection. The A-H time and H-V time for the conducted beat are 150 and 40 m sec, respectively; B. On pacing the atrium at a faster rate, the A-V block increased to 3:1, and the A-H time for the conducted beats was prolonged as a result of repetitive concealed conduction of the proximal A-V junction. The H-V time remained unchanged.

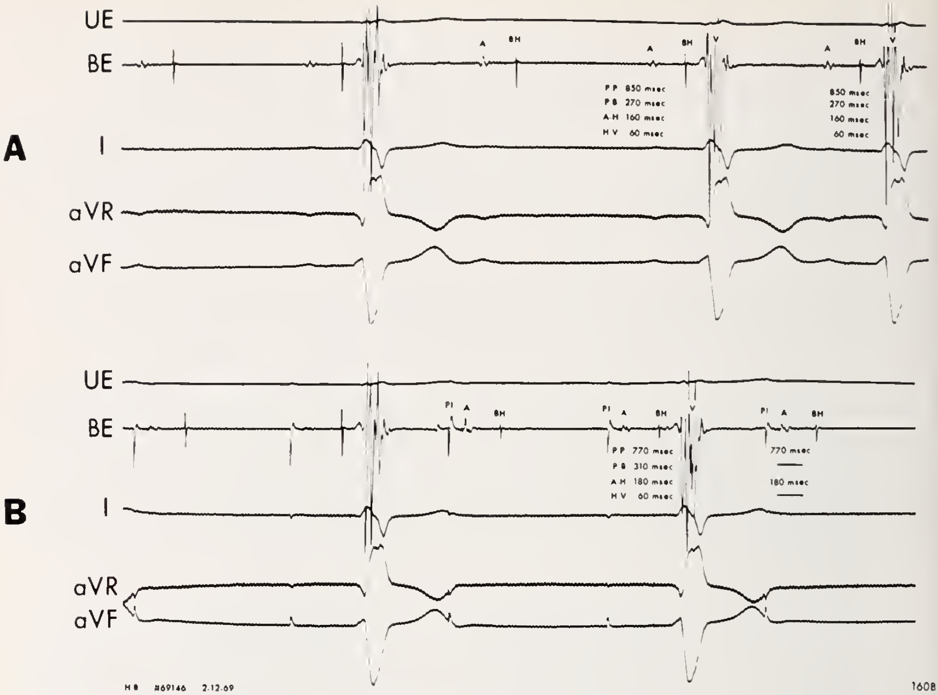
A
UE
BE
I
aVR
aVF
A
BH
A
BH
V
A
BH
V
PP 850 msec
PB 270 msec
A-H 160 msec
HV 60 msec
850 msec
270 msec
160 msec
60 msec
B
UE
BE
I
aVR
aVF
PI
A
BH
PI
A
BH
V
PI
A
BH
PP 770 msec
PB 310 msec
A-H 180 msec
HV 60 msec
770 msec
180 msec
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Fig. 9. Block in the distal A-V junction during spontaneous and paced 2:1 A-V block. The unipolar (UE) and bipolar (BE) His bundle electrogram are shown with standard ECG leads 1, AVR, and AVF. A. During sinus rhythm, atrial activity is conducted to the His bundle but is blocked below this point in every other beat, resulting in a dropped QRS complex. At a P-P interval of 850 m sec, the A-H time is 160 m sec; B. whereas with pacing (P-P = 770 m sec), the A-H time widens to 180 m sec. Although the H-V time is prolonged (60 m sec), it remains constant at any rate.

His bundle recording site, and the latter as proximal to the His bundle, or proximal to the His bundle recording site.

Results

In Table I, the measurements of the component intervals of the A-V conduction time, P-A, A-H, and H-V, are shown for 22 patients with normal P-R intervals during normal sinus rhythm (Group I). The average P-A interval was 44 m sec ± 14.0 SD of mean; the average A-H interval was 91 m sec ± 23.0 (SD); and the average H-V interval was 41 m sec ± 4.0 (SD). The average P-R interval was 176 m sec ± 24.0 (SD), at an average heart rate of 66 beats/minute ± 10.0 (SD). Figure 2(A) shows a representative example from Group I.

Right atrial pacing at rates up to 180/minute or until Wenckebach cycles occurred was performed in all patients (Groups I and II), with a progressive prolongation of P-R and A-H intervals (Figs. 2 and 3). The H-V intervals were constant at any given paced rate within the range of rates studied (Figs. 2 and 3). Qualitatively similar prolongations followed application of atrial stimuli, with varying degrees of prematurity in both Groups I and II (Fig. 4).

In the majority of cases, the H-V interval remained constant at all levels of prematurity in which the atrial stimulus was conducted through the A-V node, His-Purkinje system, and ventricular musculature. However, in several cases premature atrial beats were associated with prolongation of the H-V interval and subsequent aberration of the following QRS complex (Fig. 4). Figure 5 (A) shows an example of such aberration. In several instances, block below the His bundle occurred with premature atrial beats (Fig. 5(B)).

EFFECT OF INCREASING ATRIAL RATE IN PATIENTS WITH NORMAL P-R INTERVAL OR FIRST DEGREE A-V BLOCK

In patients with normal or abnormal P-R intervals in the basal state, atrial pacing was induced at progressively faster rates until Wenckebach periodicity occurred or a rate of 180 beats/minute was obtained. In all patients except

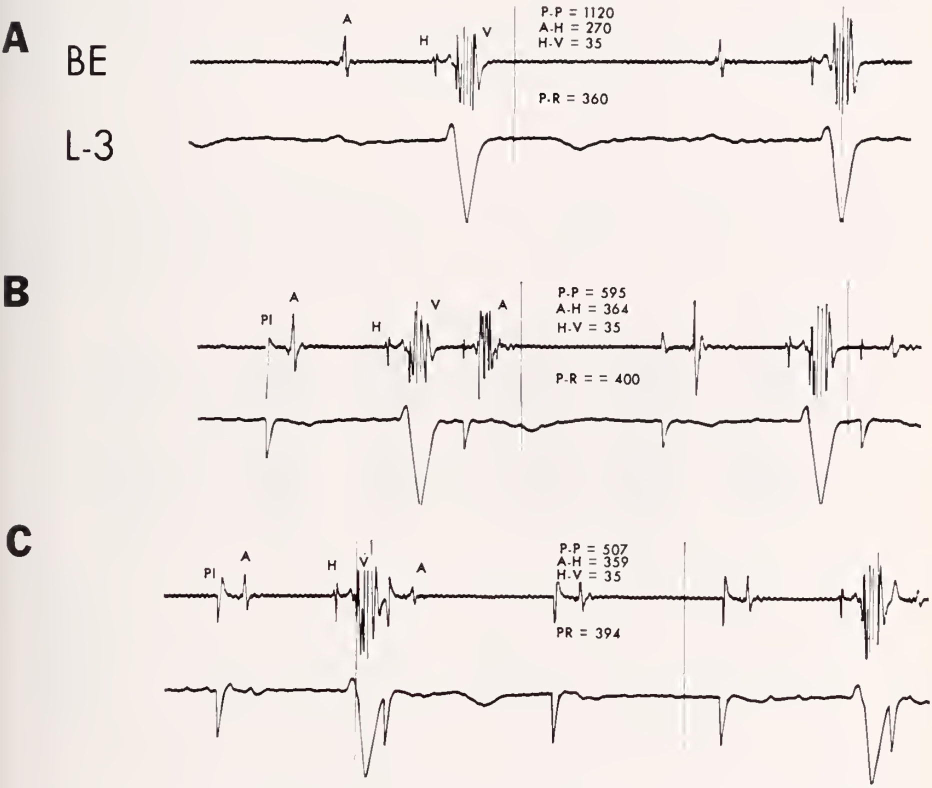


FIG. 10. The effect of atrial pacing in a patient with first degree heart block. A bipolar His bundle electrogram (BE) and standard lead III (L-3) are shown. A. During sinus rhythm at a heart rate of 54/minute, the P-R interval (360 m sec) is composed of an A-H time of 270 and H-V time of 35 m sec, respectively; B. Atrial pacing at a rate of 100/minute produces 2:1 A-V block with an A-H time of 364 m sec. H-V remains constant at 35 m sec; C. Atrial pacing at 118/minute produces 3:1 A-V block with an A-H time of 359 and H-V time of 35 m sec. In both (B) and (C) the site of block is in the proximal A-V junction. Additionally, concealed conduction into the A-V node is seen since the A-H times are longer than those seen in sinus rhythm at a faster effective rate. Interval between time lines is 1 second (1000 m sec).

one, the Wenckebach cycles showed successive increments in A-H time until the blocked beat; H-V time remained constant (Fig. 6). Another interesting form of Wenckebach is shown in Figure 7, which depicts the occurrence of Wenckebach cycles with atrial pacing during complete heart block (a fixed idioventricular rate). The block occurs below the site of His bundle recording, and thus the latter shows association with each A wave and a complete dissociation from the ventricular activity.

SECOND DEGREE A-V BLOCK

Several patients with second degree heart block were studied. The localization of these blocks could be discerned as occurring in the proximal A-V junction (Fig. 8) or distal A-V junction (Fig. 9), that is, above or below the site of recording of His bundle activity. In one patient with spontaneous second degree block at an atrial rate of 82/minute (Fig. 8(A)), atrial pacing at 115/minute produced 3:1 A-V block (Fig. 8(B)). In patients with 1:1 A-V conduction, second degree heart block could be induced by pacing at a rate, usually 10 or 20 beats, above which Wenckebach cycles developed (Fig. 10).

BUNDLE BRANCH BLOCK

Several cases of right bundle branch block and left axis deviation were studied. At least some of these cases are instances of bilateral bundle branch block. In all cases in which the electrocardiographic pattern was uncomplicated by myocardial infarction, H-V times were prolonged and ranged from

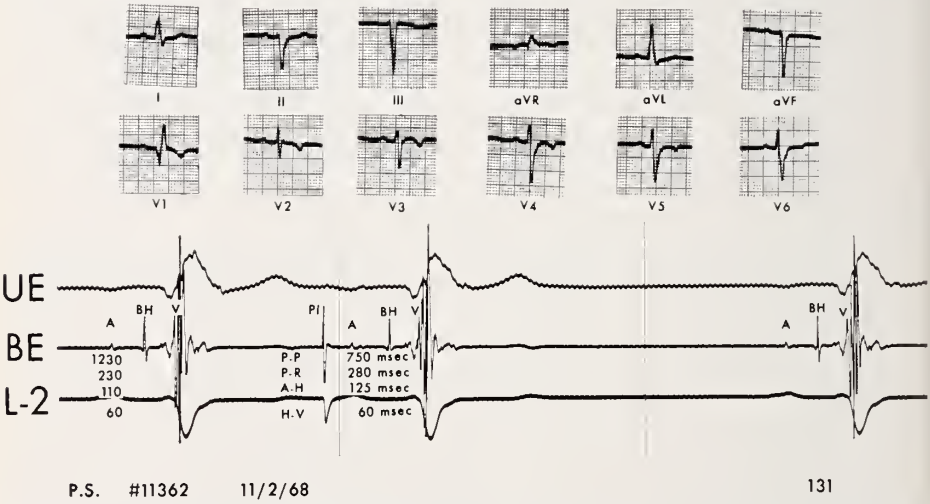
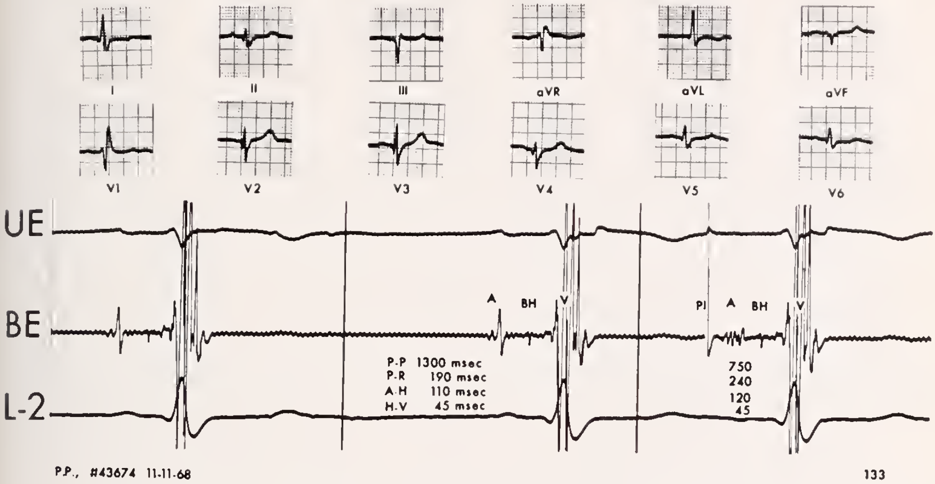


FIG. 11. A-V conduction times in a patient with right bundle branch block and left axis deviation; note leads II, III, and AVF, and lead V1, at top. A unipolar (UE) and bipolar (BE) His bundle electrogram and a standard lead 2 ECG reveal prolongation of the P-R interval in the first beat (230 m sec), composed of a normal A-H time of 110 m sec and an abnormal H-V time of 60 m sec. The second beat is an induced atrial premature beat which prolongs the A-H time to 125 m sec, while the H-V remains constant. The interval between time lines is 1 second (1000 m sec).

50–90 m sec. A representative example is shown in Figure 11. In patients with anterior wall myocardial infarction who showed this pattern, the H-V time was either within normal limits (Fig. 12), or was prolonged.


I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6
UE
BE
L-2
A
BH
V
PI
A
BH
V
P-P 1300 msec
P-R 190 msec
A-H 110 msec
H-V 45 msec
750
240
120
45
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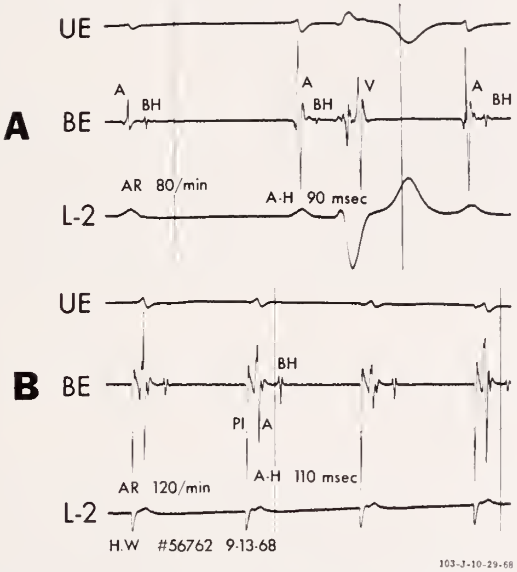
133

FIG. 12. A-V conduction times in a patient with right bundle branch block and left axis deviation, and associated anterior wall myocardial infarction (note leads I to V6, at top). The bipolar (BE) Bundle of His electrogram (BH) allows the measurement of A-H (110 m sec) and H-V (45 m sec) intervals, both within normal limits. The interval between time lines is 1 second (1000 m sec).


A
UE
BE
L-2
A
BH
A
BH
V
A
BH
AR 80/min
A-H 90 msec
B
UE
BE
L-2
BH
PI
A
AR 120/min
A-H 110 msec
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103-J-10-29-68

FIG. 13. A. His bundle recordings during complete heart block. Atrial rate (AR) = 80/minute and ventricular rate = 35/minute. The A-V block is distal to the BH deflection. The A wave and the BH deflection have a fixed temporal relationship; B. Atrial pacing at 120/minute results in a prolongation of the A-H time to 110 m sec.

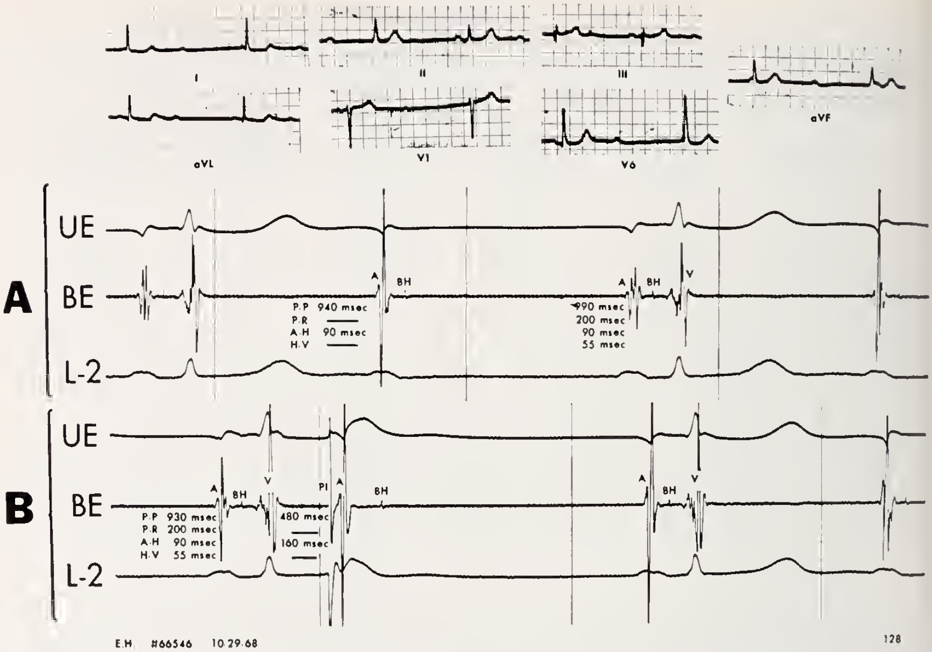

FIG. 14. His bundle recordings in a patient with complete heart block and normal QRS complexes. The standard lead electrocardiogram at the top shows complete heart block with narrow QRS complexes (90 m sec). A. During catheterization this patient showed an intermittent A-V conduction with 2:1 block. The block was distal to the site of the His bundle recording. The atrial rate is 63/minute; B. An atrial premature beat also shows block distal to the His bundle and a prolongation of the A-H time to 160 m sec. Note that the H-V time (55 m sec) is abnormal during the conducted beats.

COMPLETE HEART BLOCK

In cases of complete heart block with idioventricular rhythm, the recording of His bundle activity usually indicated block of the supraventricular impulse in the distal A-V junction (Fig. 13). In a case of complete heart block with a normal QRS complex (Fig. 14), the His bundle deflection was consistently associated with atrial activity, indicating block in the distal A-V junction, but above the bifurcation of the common bundle into the right and left bundle branches.

RECORDING OF ELECTRICAL ACTIVITY FROM THE RIGHT BUNDLE BRANCH IN MAN

In several patients, recordings from the right bundle branch were obtained. Figure 15(A) shows the recording of His bundle activity. When the catheter was advanced into the right ventricle, another sharp deflection (duration 15 m sec) was recorded 20 m sec later than His bundle deflection (duration 20 m sec) (Fig. 15(B)). In Figure 15(C) a second sharp deflection (duration 10 m sec) occurred just prior (10 m sec) to the onset of ventricular activity. The catheter electrodes at this point were further advanced into the right ventricle. All three deflections (His bundle and the two right bundle deflections) occurred during the P-R isoelectric interval of the standard lead electrocardiogram.

RECORDING OF ELECTRICAL ACTIVITY FROM THE LEFT BUNDLE BRANCH IN THE DOG

Figure 16 shows two bipolar electrograms recorded from the right heart (second trace) showing His bundle activity (duration 20 m sec), and the left heart (third trace), just inside the aortic valve, showing left bundle activity (duration 10 m sec). His bundle and left bundle activity were separated by 15 m sec. Note that both deflections occurred within the isoelectric period of the P-R interval. Postmortem examination revealed the recording electrodes in the area of the main left bundle. Figure 17 shows a bipolar recording made when the electrodes were in apposition to the upper portion of the posterior division of the left bundle branch (A) and lowest portion of this structure in the region of the Purkinje-papillary junction (B). The catheter electrode position was determined when the experiment was terminated and the heart opened with the catheter in place.

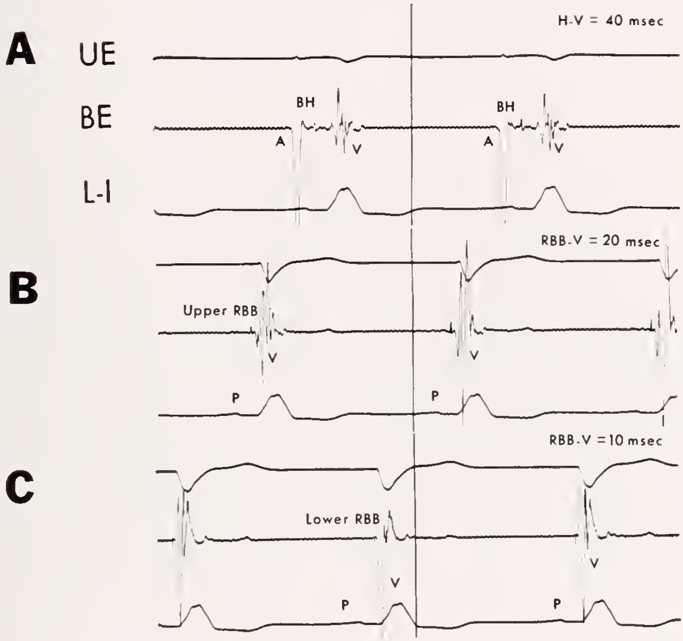

FIG. 15. Recordings from the His bundle and different parts of the right bundle branch in man. A. A unipolar (UE), bipolar (BE), and standard lead I (L-1) are shown, indicating a Bundle of His deflection (BH) of 20 m sec duration and an H-V time of 40 m sec; B. When the catheter was advanced into the right ventricle, a sharp deflection (duration 15 m sec) was seen. The right bundle to ventricular activation time (RBB-V) was 20 m sec; C. With further advancement of the recording electrodes into the right ventricle, a negative sharp deflection (duration 10 m sec) was seen with a RBB-V time of 10 m sec. Interval between time lines is 1 second (1000 m sec).

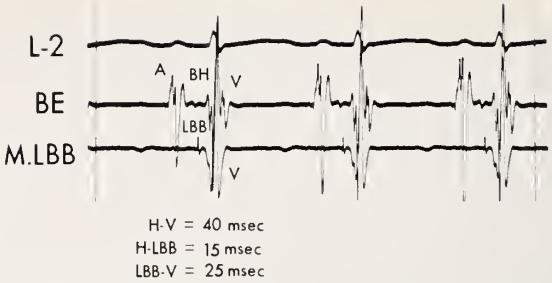

FIG. 16. Recording of His bundle and main left bundle electrical activity in the dog. The traces from above are a standard lead II electrocardiogram (L-2), His bundle electrogram (BE), and main left bundle branch electrogram (M.LBB). The His bundle activity (BH) is recorded from an electrode catheter in the right heart. Left bundle branch activity (LBB) is recorded from a similar catheter introduced just across the aortic valve through a carotid artery. The H-V time is 40 m sec and the His bundle duration is 20 m sec. The main left bundle deflection (duration 10 m sec) occurs 15 m sec later than the His bundle deflection and precedes ventricular activation by 25 m sec. The interval between the time line is 1 second (1000 m sec). Paper speed is 200 mm/sec.

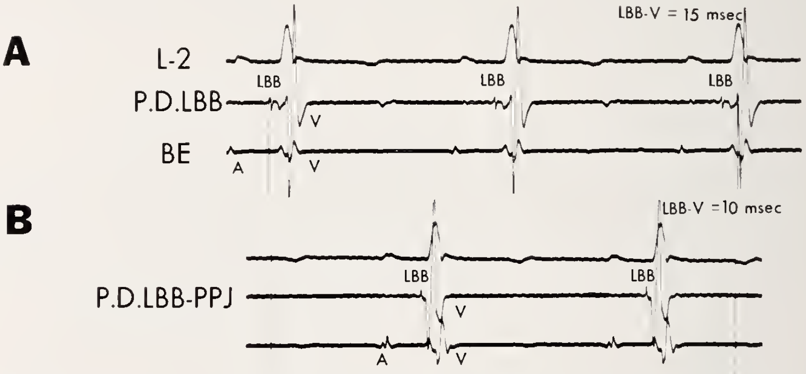

FIG. 17. Recording of electrical activity from the posterior division of the left bundle branch in dog. The traces from above are a standard lead II electrocardiogram (L-2), the posterior division of the left bundle branch (P.D.LBB), and a bipolar electrogram (BE) to show local atrial (A) and ventricular (V) activity. A. A sharp (duration 10 m sec) biphasic spike recorded from the proximal part of the posterior division of the left bundle branch is seen preceding ventricular activation by 15 m sec; B. With further advancement of the recording electrodes into the left ventricle, another deflection is recorded 10 m sec from ventricular activation. This positive spike is 5 m sec in duration. The localization of this recording site was performed postmortem in the opened left ventricle, and the recording electrodes were in opposition to the Purkinje-papillary junction (PPJ) of the posterior division of the left bundle branch and the posterior papillary muscle. Note that the left bundle branch deflections that are seen in (A) and (B) occur during the isoelectric period of the P-R interval. Paper speed is 200 mm/sec.

Discussion

HISTORICAL BACKGROUND

The standard 12 lead electrocardiogram has been useful as a diagnostic and investigational tool since its development. As has been pointed out, however, the information presented in the surface ECG is indirect insofar as impulse transmission through the A-V node and His-Purkinje system is concerned (1). In order to obtain such direct information, several recording techniques have been recently employed: (1) By the use of microelectrodes, electrical activity of single cardiac cells from different parts of the animal heart have been studied, *in vitro* (7–9); (2) In the intact animal, several investigators have examined the ventricular specialized conducting system during open heart surgery and cardiopulmonary bypass (3, 4), or during perfusion by a donor animal (10); and (3) Others have used needle electrodes (11) and wire electrodes (5, 12) to record from such specialized conduction tissue in the intact heart. Although the information obtained from such studies has added to the basic understanding of cardiac physiology, the applicability of this data to clinical situations is limited.

There have been isolated reports of recording of His bundle activity by the electrode catheter (13, 14). The consistent and stable recording of His bundle activity reported in this and other laboratories (2, 15–17) would appear to be a reliable and safe method for obtaining direct correlative data with the electrocardiogram from patients undergoing cardiac catheterization.

VALIDATION OF THE HIS BUNDLE RECORDINGS

The verification of the His bundle recording is dependent on demonstrating a dissociation of His bundle activity from atrial and ventricular activity (10). Evidence for the electrical specificity of the His bundle has been previously demonstrated in the dog during vagal stimulation (4, 5, 10), in dog and man during carotid sinus stimulation (2, 18), atrial pacing (2, 5, 10, 16), premature atrial beats with and without aberration (15, 17), and various pharmacological interventions (12, 16, 18). In the present study, the effects of atrial pacing (Fig. 2) and premature atrial beats (Fig. 5) are shown. These interventions caused A-H prolongation with or without QRS aberration (Fig. 5(A)), or the paced beats were not conducted to the ventricular musculature (Fig. 5(B)). Atrial pacing and/or the induction of premature atrial beats was performed in each patient in order to produce A-H prolongation. This effect established the independence of the recorded His bundle deflection from atrial activity.

It might be argued that the recorded "His bundle deflection" arises from the right bundle branch and not from the common bundle, since exact localization of the recording electrodes at the A-V junction cannot be ascertained even under fluoroscopy. In view of this consideration, His bundle stimulation in man, using an electrode catheter, has been performed in this laboratory (19). It was shown that during His bundle pacing, the pacing impulse to R wave interval was identical to the His bundle to ventricular activation time as measured from the His bundle electrogram. Furthermore, the shape of the QRS was unchanged. Additionally, this time interval was constant at all paced rates. Further experience with His bundle pacing may indicate that such verification is perhaps not valid in special instances, i.e., in cases of conduction defects within the His bundle itself.

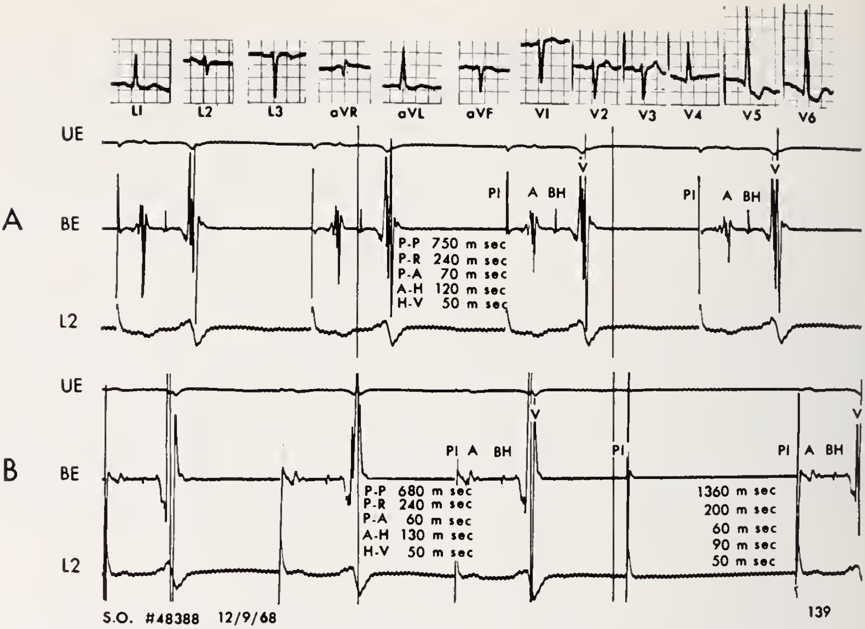
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FIG. 18. Variability of intra-atrial conduction. The traces from above are unipolar (UE) and bipolar (BE) His bundle electrogram and a standard lead II electrocardiogram (L-2). A. Atrial pacing at 80/minute shows a P-R interval (from the pacer impulse in L-2) of 240 m sec which is composed of: P-A = 70 m sec; A-H = 120 m sec; and H-V = 50 m sec; B. Atrial pacing at 88/minute indicates the same P-R interval (240 m sec); however the A-H = 130 m sec, which is consistent with the increase in heart rate. The discrepancy is due to a change in intra-atrial conduction (P-A = 60 m sec) (see text for discussion).

STANDARDIZATION OF NOMENCLATURE

Figure 1 and the section on Definition of Terms (Methods) depicts the various components of A-V conduction which are measured in our studies. In pervious studies (2) different nomenclature was used, and a standardization of terminology and the rationale for the adopted terminology is therefore appropriate. The interval from the beginning of the P wave of a standard lead 2 ECG to the His bundle deflection (P-H interval) of the bipolar electrogram was used as a measure of A-V nodal conduction time. Since the P-H time encompasses intra-atrial conduction time, changes in the P-H time may occur without changes in A-H time. Conversely, A-H time (A-V nodal conduction) may change with no change in P-H or P-R interval, due to changes in intra-atrial conduction (P-A time). An example of such a case is seen in Figure 18, in which the P-R interval remained constant at two different paced rates (Fig. 18(A) and (B)). However, the A-H time differed by 10 m sec due to changes in P-A time (intra-atrial conduction), even though atrial activity was recorded from the same site throughout.

The A-H time (see Methods, Definition of Terms) has been adopted as a better approximation of A-V nodal conduction time because: (1) The A-H measurement virtually eliminates the role of intra-atrial conduction; (2) The A wave of the His bundle electrogram is necessarily recorded close to the A-V node; and (3) The A-H measurement allows a comparison of recordings with or without atrial pacing since the site of measurement is ordinarily not altered as occurs with the P-H measurement. Probably the most accurate measurement of A-V nodal conduction time would be from the end of the A wave in the bipolar electrogram to the His bundle deflection. However, the use of such a measurement must await the elaboration of the timing and duration of the A-V nodal recording itself.

Furthermore, in other studies the H-Q interval has been suggested as a measure of conduction through the specialized conduction system of the ventricles (2, 16). However, the use of one standard ECG lead and the His bundle electrogram may not show the earliest ventricular musculature activation. This is supported by our present findings that the H-V time (see Methods), as measured using the earliest ventricular activation seen in the unipolar, bipolar His bundle electrogram, and three standard ECG leads, shows a range of 35–50 m sec (average 41 ± 4.0 m sec) in normal patients, whereas other studies have reported ranges from 33–60 m sec (16). It is therefore imperative for quantitative determinations of H-V time to employ multiple peripheral leads in conjunction with the His bundle electrogram. In this study, patients with known disease of the specialized conducting system of the ventricle showed H-V times ranging from 55–90 m sec.

CLINICAL USEFULNESS OF THE HIS BUNDLE AND BUNDLE BRANCH RECORDING TECHNIQUE

Taken in conjunction with the standard electrocardiograms, the bipolar recordings from the His bundle and bundle branches in man may be clinically significant from the following viewpoints:

(1) *Diagnostic:* The use of His bundle recordings allows for a more precise breakdown of the P-R interval (Fig. 1). The P-R interval encompasses the atrial, A-V nodal, and His-Purkinje conduction systems. The recording of a specific electrical event or events during the isoelectric P-R interval, for example the His bundle and right and left bundle electrograms, can provide direct quantitative data of conduction time, as well as direct evidence for block in fairly localized regions of the conducting system. On the basis of recording His bundle activity, various blocks have been documented which occur in the proximal A-V junction in patients with spontaneous or induced first or second degree A-V block (Figs. 6, 8, and 10), as well as in A-V block induced by premature atrial beats (Fig. 5(A) and (B)). Patients in whom A-V block occurred in the distal A-V junction have also been studied (Figs. 5(B), 9, 11, and 14). It is important to emphasize that the occurrence of focal block within the A-V node-His bundle junction, or within the His bundle itself, has been demonstrated in those cases of complete heart block showing a normal QRS pattern. A recording suggestive of such a focal block is shown in Figure 14. A patient with heart block and narrow QRS complexes revealed 2:1 A-V conduction during a His bundle study, with block in the distal A-V junction, or more likely within the lower part of the common bundle above its bifurcation. In these cases, exact localization would require the fortuitous

recording of His bundle activity on either side of a focal lesion of the common bundle.

(2) *Prognostic:* Recently several investigators have, on an empirical basis, suggested that A-V block manifesting Wenckebach periodicity subsequent to myocardial infarction would most likely not require pacemaker therapy, whereas patients exhibiting Mobitz type II block may progress to complete heart block (20, 21). The use of direct recordings from the ventricular specialized conducting system will allow a precise determination of the site of block in the clinical situations mentioned above. Wenckebach periodicity may occur within the His bundle in some cases (unpublished observations); in cases of 2:1 block with a normal QRS complex, the block may be located in the proximal A-V junction (Fig. 8).

The present study also shows a clear distinction between the H-V time of the patients with normal A-V conduction (average 41 ± 4.0 SD), and those patients with right bundle branch block and left axis deviation (H-V range 55–90 m sec) without electrocardiographic evidence of myocardial infarction. Does the existence of a prolongation of H-V time in the latter group represent the early stages of bilateral bundle branch block with a high incidence of subsequent complete heart block? Follow-up studies of such patients are now being carried out in this laboratory. In this regard, it is important to emphasize that only through the direct His bundle recording can one determine the absence or presence of prolongation of conduction time in the ventricular specialized conducting system in those patients showing right bundle branch block and left axis deviation and associated myocardial infarction. In these cases, the left axis deviation may be a result of extensive myocardial damage, and not due to primary conduction system disease.

Another important prognostic consideration in the localization of A-V blocks is the use of atrial pacing and/or premature atrial stimulation in conjunction with His bundle recordings. Atrial pacing can test proximal A-V junction conduction. Additionally, premature atrial stimuli can produce prolongation of H-V time or block distal to the site of His bundle recordings (Fig. 5(B)). The prognostic value of such "stress tests" of the A-V conduction system remains to be determined.

(3) *Theoretical:* Direct recordings from the A-V conduction system may be useful in obtaining crucial evidence regarding electrophysiological hypotheses based on indirect electrocardiographic interpretation. For example, the finding of a consistent H-V prolongation in those patients with right bundle branch block and left axis deviation without myocardial infarction would appear more compatible with the concepts of bilateral bundle branch block than right bundle branch block and left anterior hemiblock.

RECORDINGS FROM THE RIGHT AND LEFT BUNDLE BRANCHES

A recording from the right bundle branch in man has been recently reported using the electrode catheter technique (16). Our recordings show similar qualitative and quantitative characteristics, and correlate well with previous direct recordings in animals (1, 3, 4). The recordings from the left bundle branch in the dog, using the electrode catheter technique, would tend to indicate that such recordings might be accomplished in man during retrograde left heart catheterization. The recordings from the right and left bundle branches should provide further localization and quantitation of block occurring in the distal A-V junction, as well as pertinent quantitative data in regard to conduction through the specialized ventricular conduction system in normal and diseased hearts.

Summary

In the present study, qualitative and quantitative data of conduction times through the component parts of the A-V junction are reported in 100 patients with normal conduction and various A-V conduction defects. The measurement or conduction times during the P-R interval is made possible by a newly developed technique using a local bipolar His bundle recording in conjunction with three standard limb leads during routine right heart catheterization.

Normal values for intra-atrial conduction (P-A time = 44 m sec ± 14 SD), A-V nodal conduction time (A-H time = 91 m sec ± 23.0 SD), and His-Purkinje system conduction time (H-V = 41 m sec ± 4.0 SD) have been established in 22 patients at an average heart rate of 66/minute ± 10.0 SD. Similar measurements in patients with right bundle branch block and left axis deviation without associated myocardial infarction consistently revealed a significant prolongation of H-V time (55–90 m sec). The use of the His bundle recordings to localize the site of block in various A-V conduction defects including first, second, and third degree heart block and bundle branch blocks are illustrated. Recordings from the right and left bundle branches in man and dog are also presented.

The development, validity, terminology and clinical use of direct intracardiac recordings from the specialized conduction system of the heart are discussed.

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Redistribution of the Cardiac Output during Alterations in Blood Gas Levels*

F. HAAS, M.S., AND E. H. BERGOFSKY, M.D.

Changes in O_2 and CO_2 levels of blood have long been known to influence the blood flow and the vascular resistance of various regions and organs in all mammals, including man (1–4). In fact, in some organs or in some views (5), the gaseous levels of the perfusing blood or tissues are regarded as the major regulators of regional blood flow; as a result, the reactivity of the circulation of individual organs has been extensively studied and reported. In the main, these studies have used isolated perfused organ systems, such as the excised extremity, or a single organ studied in the intact animal. Because, perhaps, of the nature of these preparations, the results of the studies have often varied both quantitatively and qualitatively.

The present study differs from previous ones in that it is designed to investigate the effects of changes in blood gas levels on the distribution of the cardiac output to the important and accessible organs of the intact mammal. We organized the study to take cognizance of the fact that the effects of alterations in O_2 and CO_2 levels on organ perfusion may be mediated: (1) directly, at the level of the small resistance blood vessel; (2) by reflex effects via the sympathetic nervous system supplying vasoactive fibers to blood vessels; (3) by alterations in systemic blood pressure preceding from a variety of causes. Regardless of the mechanism, the present investigation deals with the integration of a variety of factors in the redistribution of the cardiac output during hypoxia, hyperoxia, hypercapnia, and hypocapnia.

Methods

The general procedure involved the measurement of cardiac output, systemic blood pressure, regional blood flow, and arteriovenous differences in blood gases for five different important organs during the experimental increases and decreases in blood O_2 and CO_2 levels in relatively intact animals.

For this purpose, 24 mongrel dogs, weighing from 15 to 30 kg, were anesthetized with an intravenous injection of sodium pentobarbital, 30 mg/kg. An endotracheal tube was inserted so that both tidal volume and frequency of ventilation could be controlled by a positive pressure Harvard respirator pump, and so that appropriate mixtures of O_2 and CO_2 could be administered as inspired air from this pump in order to change the blood gaseous composition. Polyethylene catheters were also inserted into the trachea, into the femoral artery, and floated from the jugular vein into the pulmonary artery.

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Pressures were measured from these areas by means of Statham strain gauges and continuously recorded on a polygraph. In order to measure regional blood flow and arteriovenous differences for blood gases, the major artery supplying an organ and the major vein draining it were exposed by surgical procedures, an electromagnetic flow probe placed about the artery, and an indwelling polyethylene catheter inserted into the vein.

The femoral artery, which was chosen as the arterial supply of the limb region, representing mainly muscle and skin, and the femoral vein were easily dissected out bilaterally for both hind limbs; in one of the extremities, both the femoral and sciatic nerves were cut in order to compare the effects of hypercapnia on both an intact and denervated muscular extremity. For the kidney, the renal artery was exposed through an incision in the lumbar region, and venous samples were obtained by means of a No. 60 polyethylene catheter sutured into the renal vein. In the case of the spleen, the splenic artery was exposed by means of a midventral incision as it branched from the celiac artery; venous samples were taken from a polyethylene catheter inserted into one branch of the splenic vein. In the case of the bowel, the cranial mesenteric artery was exposed by means of an abdominal incision; venous samples were obtained from a catheter inserted into one of the arcade veins and threaded into the mesenteric vein. For the brain, the vertebral artery was isolated as it branched from the subclavian artery in the pocket of a neck incision; venous blood was obtained from a catheter passed craniad through the external jugular vein until it reached the level of the jugular fossa of the skull.

The experiments were of four types: hypoxia, hyperoxia, hypercapnia, and hypocapnia. In the first three types of studies, the experimental conditions were produced by substituting a breathing mixture containing various gas mixtures for the regular room air being delivered by the Harvard pump. These special breathing mixtures included 10% O_2 (balance N_2) to produce hypoxia, 100% O_2 to produce hyperoxia, and 5% CO_2 (balance air) to produce hypercapnia. Hypocapnia was induced by hyperventilating the animal. The order of the experiments was randomized in order to control the possibility of progressive deterioration of vasoactive responses during the course of the anesthesia; not all of the changes in blood gases were imposed on the same animal. The experimental periods lasted 20 minutes, and were sandwiched between 20 minute control periods breathing room air.

TECHNIQUES

The cardiac output was measured in half the animals by means of the Fick principle, with care to insure that a steady state of respiration and circulation had been reached prior to the withdrawal of blood and air samples. In the other half of the animals, the cardiac output was measured both by the Fick principle, and by means of an electromagnetic flow probe encircling the main pulmonary artery. For this latter purpose, the pulmonary artery was exposed by means of a left thoracotomy through the fifth intercostal space; prior to measurements, however, the chest was closed and the intrapleural air was withdrawn. The electromagnetic flowmeters and probes (Medicon, Division of Statham) were calibrated by a system incorporating freshly excised segments of arteries on which were mounted the flow probes, and through which isotonic saline was perfused at a known rate by a Sigmamotor pump.

At the end of each experimental or control period, samples of systemic arterial and pulmonary arterial blood were withdrawn in heparinized syringes over a 30 second period, while a 1 minute collection of expired air was made into Douglas bags. The O_2 and CO_2 tensions of the blood were measured by a Clarke O_2 electrode and Severinghaus CO_2 electrode, respectively, and metered by a Beckman, Model 150, gas analyzer. This meter was calibrated by known gases which had been analyzed by the microScholander technique. The blood pH was measured separately by a capillary glass electrode, utilizing a Radiometer pH meter. From the O_2 tension and pH of the blood, the O_2 saturation was calculated and, from the hematocrit performed by the Wintrobe technique, the O_2 concentration in both arterial and venous blood was calculated. The O_2 and CO_2 tensions of expired air were continuously collected and analyzed by an infrared CO_2 analyzer (Godart) and by a Beckman paramagnetic meter.

Results

A typical recording of monitored pressures and flows is shown (Fig. 1) during an experiment correlating splenic and brain blood flow with cardiac output

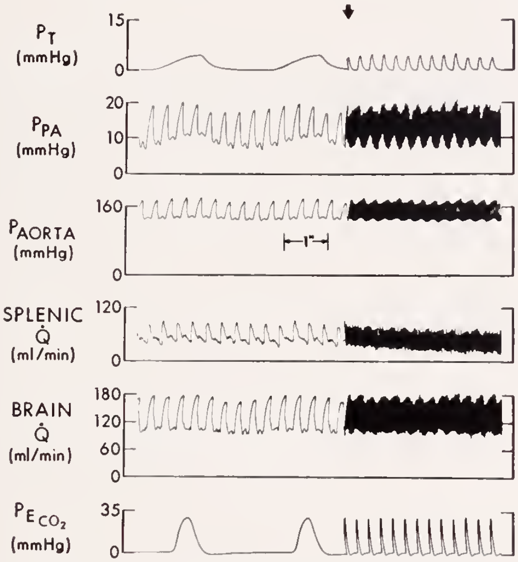

FIG. 1. *Pressure pulses in the pulmonary artery* (P_{PA}) *and the aorta* (P_{AORTA}) *matched with splenic and brain blood flow* ($\dot{Q}$) *measured by electromagnetic flowmeters in an anesthetized dog.* Also shown are the tracheal pressure (P_T) and the expired or end-tidal CO_2 tension ($P_{E_{CO_2}}$). In this figure, the polygraph paper is run at first on fast speed to demonstrate the synchrony of the flow pulses with the pressure pulses and the fact that electromagnetic flowmeters are yielding a pulsatile tracing; at the arrow, the paper speed is decreased in order to record an entire experiment with changes in blood gas.

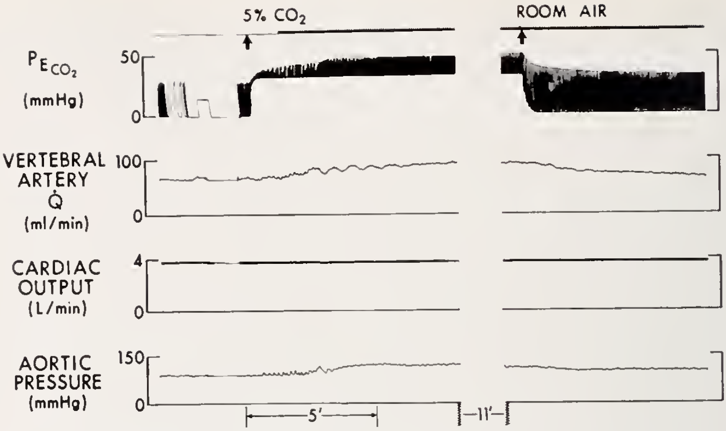

FIG. 2. *A continuous recording of vertebral arterial flow, cardiac output (pulmonary arterial flow), and aortic pressure during 5% CO_2 breathing.* The onset of 5% CO_2 breathing is shown at the arrow, at which point the end-tidal carbon dioxide tension abruptly rises to a new plateau. As indicated, vertebral arterial flow rose by approximately 30 percent without any material change in the cardiac output. Following the resumption of room air breathing, the vertebral arterial flow returned to its previous control value.

and systemic vascular resistance. As indicated in this figure, after implantation of the electromagnetic flow probes, measurements of blood flow to organs, such as the spleen and the brain, were considered acceptable only when pulsatile and synchronous with the aortic pressure pulse. Also shown on this tracing are tracheal pressure, pulmonary artery pressure, and end-tidal carbon dioxide levels. In Figure 2, data are shown during a typical experiment in which brain blood flow was measured through an electromagnetic flow probe implanted on the vertebral artery, after ligation of the internal carotids, during 5% CO_2 breathing. As indicated by the arrow, the onset of hypercapnia produced a 30 percent increase in vertebral arterial flow in this particular experiment, with a 10 percent increase in aortic pressure, and no change in cardiac output. All of these parameters returned to their control values when the breathing of room air was resumed.

The effect of altered blood gases on total and regional blood flow and vascular resistance is shown in Figures 3 and 4, respectively:

(1) *Hypercapnia:* The quadrants labelled hypercapnia in both these figures indicate that blood flow rose and vascular resistance fell in all regions except the limbs. When the nerve supply to the limbs was cut, hypercapnia then elicited a rise in flow and fall in resistance in this particular region. It is also apparent that the brain and bowel enjoy the greatest degree of vasodilation (approximately 30 percent), whereas the spleen, the kidney, and the denervated limb increase their blood flow from the insignificant value of 5 percent in the kidney, to the modest level of 15 percent for the spleen. The data also indicate that, for the whole animal, the rise in cardiac output of 10 percent went hand in hand with a rise in systemic blood pressure, and total peripheral resistance was largely unchanged. In summary, during hypercapnia, blood from the skin and/

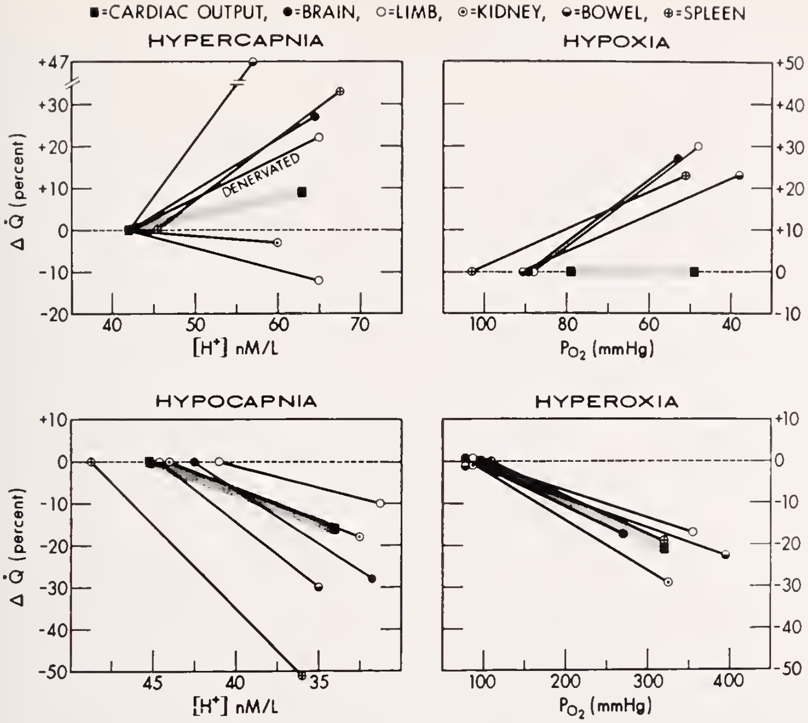

Fig. 3. *Effect of hypercapnia, hypoxia, hypocapnia, and hyperoxia on regional blood flow and cardiac output.* In the case of hypercapnia and hypocapnia, the percentile change in cardiac output ($\Delta\dot{Q}$) is plotted against the actual change in hydrogen ion concentration. In the case of hypoxia and hyperoxia, the percentile change in cardiac output is plotted against the arterial oxygen tension. All control values begin at zero on the ordinant, and thus the control values for arterial hydrogen ion concentration or arterial oxygen tension appear, along with the experimental values during the various physiological conditions imposed.

or muscle bed of the limb is diverted mainly to the brain and bowel, with smaller increments in blood flow going to the spleen.

(2) *Hypoxia:* As indicated by the panels on hypoxia in Figures 3 and 4, cardiac output and total systemic vascular resistance were not altered by hypoxia; but, as Figure 3 shows, the bowel, spleen, brain, and limb all showed increases in blood flow, ranging from 20 to 34 percent greater than control values. These were accompanied by corresponding decreases in the individual regional vascular resistance, although, in some individual groups of animals, part of the increased blood flow was also attributable to increases in blood pressure. A particular example of this response is shown by the limb, where blood flow increased by 34 percent, but the vascular resistance of the limb dropped by only 12 percent, and by the bowel, where the increase in blood flow of 23 percent was accomplished entirely by rises in blood pressure, since no decrease in regional vascular resistance for the bowel occurred.

(3) *Hypocapnia:* The results of hypocapnia represent a special situation in that both cardiac output and systemic vascular resistance decreased simul-

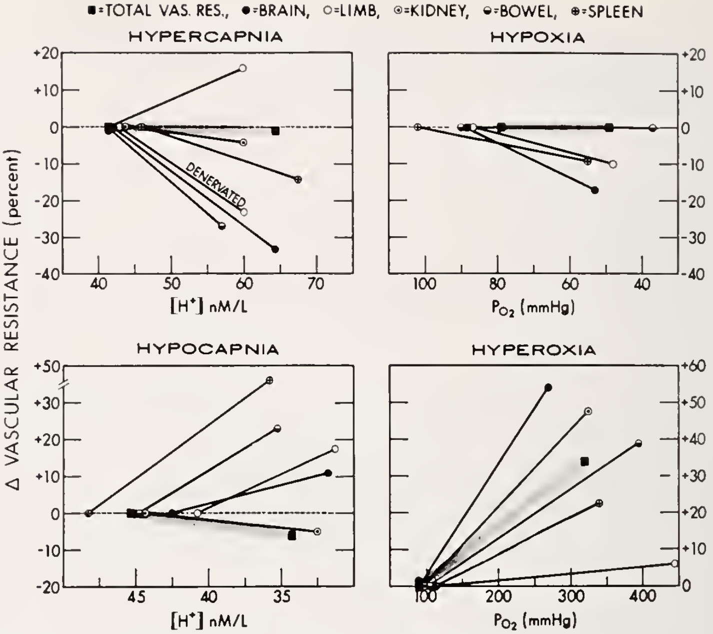

Fig. 4. *Effect of changes in the blood gases on total and regional vascular resistance.* The form of this figure is similar to that of Figure 3, except that the percentile change in vascular resistance is plotted against the actual values for hydrogen ion concentration during hypercapnia and hypocapnia, and against the actual values for arterial oxygen tension during hypoxia and hyperoxia.

taneously. Such a response is most likely due to decreased venous return into the thorax, owing to the high intrathoracic pressure imposed by the Harvard respiration pump during the process of hyperventilation. The fall in cardiac output, however, averaged only 16 percent. During the process, the spleen evinced the greatest increase in vascular resistance (+46 percent), and the bowel the next greatest increase in vascular resistance (+23 percent), with appropriate decreases in the respective regional blood flows of these two organs.

(4) *Hyperoxia:* As Figures 3 and 4 indicate, hyperoxia produced a consistent decrease in cardiac output and blood flow to all the regions studied, ranging from 18 to 33 percent. Increases in total vascular resistance and of each regional vascular resistance during hyperoxia were also consistent. However, the vascular resistances of the brain showed the greatest increase, averaging 55 percent greater than the control values, closely followed by renal vascular resistances, which increased by an average value of 49 percent. Both these increases considerably exceeded the increment in total peripheral vascular resistance, which was only 35 percent greater than the control value. At the other end of the scale, the limb, bowel, and spleen showed much smaller increases in their regional resistances; the increases in these organs averaged between 25 and 30 percent. Thus, the rise of total peripheral vascular resistance of 35 percent was the result of an approximate average of the vascular resistance of

organs, such as the brain and kidney, with intense vasoconstriction during hyperoxia, and of organs, such as the limb and bowel, with only moderate degrees of vasoconstriction.

Discussion

The experience with hypercapnic acidosis in the intact animal is of particular interest in that it indicates not only the relative degree of vasodilation of each of the regions during hypercapnia, but also the origin of blood required to perfuse the vasodilated vascular beds: (1) Of the major organs studied, the brain, followed by the bowel, are the two with the maximal sensitivity to hypercapnic acidosis; the spleen and kidney enjoyed only minor degrees of vasodilation; (2) The cardiac output itself rose only slightly during hypercapnic acidosis, and therefore did not constitute the major source of blood supply to the organs with vasodilated vascular beds in this particular animal preparation, and; (3) The vasoconstrictor response in the intact limb during hypercapnic acidosis suggests that at least one source of increased blood flow for the vasodilated beds is the muscle and skin.

Thus, in the anesthetized dog, the systemic vascular response to hypercapnic acidosis is accomplished, in the main, by a redistribution of regional blood flows. In man, where somewhat larger increments in cardiac output may occur during acidosis, as an inconstant observation (6), the source of the increased regional blood flows may differ slightly in that somewhat greater amounts of blood supply to dilated regional circulations may come from the higher cardiac output.

The present experience with a denervated limb offers an explanation for the disparate performance of the regional blood vessels of the limb and the remainder of the organs studied. It suggests that the basic response of the non-innervated blood vessel to hypercapnic acidosis is one of vasodilation; consequently, vascular beds, such as those of the brain and possibly bowel, which have only rudimentary sympathetic innervation, will manifest maximal degrees of vasodilation; whereas those regions with high degrees of sympathetic innervation, such as the skin and muscle, will manifest vasoconstriction. The latter may be expected as a result of the decreased systemic blood pressure activating baroreceptors which, in turn, will act to cause vasoconstriction wherever the sympathetic innervation is most concentrated. In this regard, the splanchnic vascular bed appears to behave differently from that of muscle and skin: Whereas, the splanchnic bed is supplied with sympathetic vasoconstrictor fibers, it dilated in response to hypercapnia, despite apparent activation of baroreceptors during decreases in systemic blood pressure; such a response suggests that this vascular bed may have only a few vasoconstrictor fibers or fibers which may not be activated in response to baroreceptor stimulation.

The conclusion that the basic response of the vascular beds to hypercapnic acidosis is vasodilation has ample support from previous studies. For the brain, these include those of Kety and Schmidt (7), who noted a 33 percent increment in cerebral blood flow during 5% CO_2 breathing, those of Lenox and Gibbs (8), and those of Shapiro, Wasserman, and Patterson (9) in man; in a similar manner, for the bowel, Sidkey and Bean (10) also found increases in blood flow during hypercapnia and, finally, Bronk and Gessell (11) noted the contradictory rise in carotid flow and fall in femoral arterial flow during hypercapnia. Our conclusion that the direct effect of hypercapnia on vessels is vasodilation, regardless of the region or organ, is also suggested by such studies on isolated arterial segments as those of Carrier et al (12), Fleishman, Scott, and Haddy (13), and Coursert et al (14).

Hypoxia mainly produced a decrease in vascular resistance and an increase in blood flow in the regions tested. As in previous studies in animals (15), the cardiac output was unchanged. In a similar manner to the results with hypercapnia, the brain led all the rest of the organs in increasing its blood flow and decreasing its vascular resistance. However, hypoxia differs from hypercapnia in one respect: The limb vasodilated during hypoxia like the rest of the organs tested, rather than vasoconstricted; this difference in performance could be explained by the hypothesis that the direct vasodilatory effect of hypoxia on blood vessels of the limb overcomes the reflex sympathetic vasoconstrictor effects on these same vessels set off by baroreceptors responding to generalized vasodilation. The blood required to supply the vascular beds of the vasodilated organs must have come from some other uninvestigated vascular bed which underwent vasoconstriction: The most likely vascular bed is the renal circulation, which was not studied in this particular group of animals because, in handling the kidney in order to adjust flow probes early in the course of this investigation, intense renal vasoconstriction precluded adequate studies of the renal circulation. However, previous data, which have been recently summarized (16), indicate that renal vasoconstriction is a usual observation during hypoxia.

With respect to hypocapnia, where decreases in cardiac output (due to mechanical hyperventilation) were combined with the direct effects of hypocapnic alkalosis on blood vessels, all organs evinced a more or less similar degree of vasoconstriction. It is of special interest that the brain, when studied in the same manner as the rest of the organs, did not show a specially intense degree of vasoconstriction to this stimulus.

The present experience with hyperoxia and previous data (17) indicate that the circulation to the brain is extremely sensitive to high arterial oxygen levels, followed by that of the bowel and kidney. Other organs, such as the spleen and the limb are less sensitive, but no organ which we studied failed to evince vasoconstriction and decreased blood flow. As a result, it is not surprising that cardiac output fell, and that this decrease is most likely attributable to slowing of the heart by baroreceptors detecting the rises in blood pressure associated with generalized increases in vascular resistance.

It is apparent that the hyperoxic stimulus is an extremely effective one: It produced consistent vasoconstriction despite the fact that the increased arteriovenous differences for O_2 and CO_2 (25 percent greater than control) that occurred because of decreased regional blood flows must have been accompanied by rises in tissue and venous blood PCO_2 and hydrogen ion concentration. The latter vasodilator stimuli would be expected to counteract the hyperoxic vasoconstrictor stimulus, even though the automatic respirator held arterial hydrogen ion concentration and PCO_2 constant. That the net effect of the experimental procedure was vasoconstriction suggests either: (1) that this type of experiment tends to underestimate the effectiveness of the vasoconstrictor response to hyperoxia; or (2) that hyperoxia acts mainly locally on the arterial or arteriolar segment of the regional blood vessel, which is far less affected by rises in tissue and venous hydrogen ion and PCO_2 levels than the venous segments or the precapillary segments of the capillary bed.

Summary

The redistribution of the cardiac output to five important organs (brain, bowel, limb, spleen, and kidney) has been studied after induced alterations in the blood gaseous composition in the anesthetized mammal.

For the conditions of hypercapnia and hypoxemia, the general effect was vasodilatory, with the brain enjoying the greatest degree of vasodilation, followed closely by the bowel. Since cardiac outputs were generally unchanged, some organs were required to donate blood during hypercapnia and hypoxemia, and these were most often the skin and/or muscle of the limb. This hierarchical distribution of the cardiac output, whereby important organs increased their perfusion rate, under stressful situations, at the expense of other organs, seems to be inversely proportional to the degree of autonomic innervation of the vascular beds of the individual organs. Thus, the direct effect of hypercapnia and hypoxemia on vascular beds is one of vasodilation, but the ensuing fall in systemic blood pressure works through baroreceptors to cause vasoconstriction in those organs most richly supplied by vasoactive autonomic fibers; the present data indicate that hypercapnia and hypoxemia act directly on the cerebral circulation by vasodilation, but that the vasodilatory effects of these stimuli on other organs are wholly or partially overwhelmed by sympathetic vasoconstriction.

Hypocapnia and hyperoxia elicit less complicated hemodynamic reactions than those of hypercapnia and hypoxemia in that generalized decreases in total and regional blood flows to organs occur, along with general increases in regional vascular resistances.

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Support of the Circulation In Acute Myocardial Infarction*

LESLIE A. KUHN, M.D.

Rationale of Circulatory Support in Acute Myocardial Infarction

The clinical syndrome of shock following acute myocardial infarction is associated with very high mortality (averaging about 80 percent), despite a variety of medical regimens. It occurs in about 10 to 15 percent of all hospitalized patients with acute myocardial infarction. Indeed, with the wide employment of coronary care units which have resulted in the successful prophylaxis and treatment of significant arrhythmias, the shock syndrome is responsible for most of the deaths in hospitalized patients with acute myocardial infarction. When it is realized that there are several hundred thousand patients with acute myocardial infarction hospitalized in the United States each year, and even with coronary care units there is a current hospital mortality of about 18 percent, it is apparent that systems of circulatory support which may reduce mortality rates from shock even a relatively small amount, may nevertheless result in saving a large number of lives.

Since there is not adequate information available as to the ultimate effects of systems of mechanical circulatory support in humans with acute myocardial infarction with shock, the question may be asked whether any technique, even if it could be proved safe and feasible to apply in such critically ill patients, can be efficacious, or whether such profound myocardial destruction and/or circulatory deterioration is associated with the shock syndrome that no system of temporary circulatory support could reasonably be expected to result in cure of the patient. Sufficient critical data to answer this question are not available to form a firm idea of the potential value of such a system, but there is some pertinent information which suggests that appropriately applied systems of circulatory support may well be expected to lower mortality substantially in acute myocardial infarction with shock.

In support of this view are some clinical data, discussed subsequently, of at least one system of circulatory support applied to patients with acute myocardial infarction with shock refractory to conventional medical management. In addition, although the shock syndrome is usually associated with large areas of infarction, it is of interest that careful pathological studies do not uniformly show areas of myocardial infarction (1) or coronary arterial occlusion (2) that are more extensive than those found in patients who do not develop shock. In fact, in the latter study, shock occurred with significantly greater frequency in patients with branch occlusions (particularly branches

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of the left circumflex coronary artery), than in those with main stem coronary arterial occlusions. This suggests the possibility that at least a portion of the myocardial infarctions seen in some of these patients may be a result of continuing arterial hypotension with resultant diminutions of coronary flow. Therefore, it is rational to adopt the view that the patient in shock, at least relatively early in the syndrome, may not have such extensive coronary occlusion or myocardial infarction that effective use of circulatory support is precluded.

Physiological Aims

One of the difficulties in determining rational physiologic aims to be met by a circulatory support device in acute myocardial infarction with shock is that hemodynamic data have not been collected in sufficient numbers in these patients to determine sequential alterations or the precise prognostic value of specific hemodynamic abnormalities. In addition, it is evident from the studies that are available that, even though patients with acute myocardial infarction with shock may present a common clinical syndrome, their hemodynamic derangements have shown considerable variations among different patients. For example, all patients with the shock syndrome (systolic arterial blood pressure of 80–90 mm Hg or less), by definition, will also demonstrate cold skin and oliguria, and most will also exhibit some degree of mental obtundation. Therefore, statements have been made that these patients have undergone maximal systemic vasoconstriction, thus obviating the need for vasopressor drugs in their therapy. However, analysis of total systemic vascular resistance indicates variations in response, with some patients showing elevated, others normal, and a few, diminished, total systemic vascular resistance, despite reduction of the cardiac output in all of these groups. What is apparent in these patients is increased regional vasoconstriction affecting the kidneys and skin. Despite the increased regional vasoconstriction, in many patients vasopressor therapy may result in further total systemic vasoconstriction, with resultant elevation of coronary perfusion pressure and improvement of coronary flow.

Although the shock syndrome is associated with a precipitous fall of cardiac output following acute myocardial infarction, it does not appear to be due solely to "congestive heart failure" in many instances. In some studies, experimental (3) and clinical (4), it has been demonstrated that the cardiac output may fall equally in animals or humans with acute myocardial infarction, who do or do not experience shock, suggesting that factors acting on the peripheral vasculature may be of some importance in the genesis or maintenance of the arterial hypotension in this syndrome. While clinical congestive heart failure may precede, coexist with, or follow the shock syndrome in acute myocardial infarction, there are patients with shock who have none of the usual clinical signs of congestive heart failure. In reported patients with central venous or right atrial pressures, normal values were obtained in about one-third. This does not imply that ventricular function is normal, since right-sided pressures may not invariably reflect alterations of left ventricular diastolic pressure. Indeed, those few measurements of left ventricular pressures which have been performed (5, 6) have indicated almost uniform elevation of diastolic pressure in the presence of shock. Nevertheless, the patient may have clear lungs, flat neck veins, and be free of dyspnea or orthopnea. He need not present the clinical syndrome of severe volume overloading of the left ventricle.

Many investigators of systems of circulatory support in acute myocardial infarction with shock have stressed the necessity to reduce "left ventricular work," and thus the oxygen requirement of the left ventricle. This has some rationale inasmuch as any acutely ischemic condition results in a disproportion between the oxygen needs and available blood flow to the organ. For the purpose of reducing left ventricular oxygen requirement in acute myocardial infarction with shock, two main techniques have been investigated: left ventricular bypass, and "counterpulsation." The latter is achieved by withdrawal of blood from the aorta, and hence reduction of left ventricular "pressure work" (an important determinant of left ventricular oxygen requirements) in systole, and infusion of this blood into the aorta in diastole, at a phase of maximal coronary flow. In treating the patient with acute myocardial infarction with shock, shall our principal aim be to reduce the left ventricular oxygen requirement? Analysis of clinical experience in this syndrome and of our own experimental results suggests that this is an insufficient principle upon which to base therapy. If this is accomplished by bypass of the left ventricle, it requires large volumes of bypass flow to reduce left ventricular oxygen requirement, probably beyond the capacity of most closed-chest systems to deliver, inasmuch as a high percentage of the left ventricular volume must be shunted from it to materially reduce its oxygen needs (7). If it is decided to reduce left ventricular oxygen requirement by means of drugs by reducing the systolic pressure against which the left ventricle must pump, one achieves this by agents which result in substantial reduction of an already diminished coronary flow, the fundamental determinant of which is central aortic perfusion pressure. If it is achieved by counterpulsation of blood, our own experimental results have demonstrated little improvement of the diminished coronary flow or anaerobic myocardial metabolism (8) when there is significant aortic hypotension, primarily because the coronary perfusion pressure remains inadequate, even though it is "phased" optimally. Other experiments have also indicated little diminution of systolic pressure with this technique when used during significant hypotension (9). In addition, clinical experience has failed to substantiate the advantages of reduction of left ventricular pressure work in affecting cure of myocardial infarction with shock. Patients whose arterial pressure cannot be raised substantially from shock levels invariably succumb. The patients who are treated successfully invariably have sustained elevations of arterial pressure as an important part of their clinical response. It can be readily appreciated that reduction or failure to increase a low arterial pressure will be associated with further diminution of coronary flow, myocardial anaerobic metabolic alteration, deterioration of cardiac function, increased susceptibility to significant arrhythmias, further reduction of cardiac output, and acidosis. These lead to further aortic hypotension which sustains the grievous cycle of deleterious reactions.

The use of hypothermia, which can effect an appreciable reduction of myocardial oxygen consumption (if unaccompanied by substantial shivering), is probably limited by the same threshold for the development of ventricular fibrillation or asystole in patients with acute myocardial infarction, as in those without infarction (10). Therefore, at most, it would probably be utilized as an adjunctive technique when combined with other methods, but can be expected to be of limited benefit when used alone.

While some of the devices which have been advocated to reduce left ventricular oxygen requirement in acute myocardial infarction with shock may be useful in the treatment of this syndrome, we believe their efficacy rests on other physiological principles. What should these principles be? Our investigations to date indicate that they should be twofold, neither resting on the inherent assumption that the left ventricular oxygen requirements must be reduced, although this would be, of course, a useful by-product of the application of any circulatory assistance device.

One fundamental principle consists of reducing severe volume overloading of the left ventricle, with its attendant life-threatening pulmonary edema; because of accompanying tachycardia, increase of cardiac size, and increase of circulating catecholamines, severe volume overloading increases myocardial oxygen requirement. While techniques to accomplish this may at times also reduce the oxygen consumption of the infarcted left ventricle, it is not necessary to accomplish this to achieve beneficial results. While bypass of most of the left ventricular blood may be necessary to reduce left ventricular oxygen consumption, circulation through an assist device of only about 25 percent of volume flowing through the left ventricle may effectively reduce elevated left atrial pressure (7).

The second fundamental principle should be to increase the oxygen supply to the acutely ischemic myocardium, even though this may mean an increase of left ventricular pressure work. There are two feasible methods of achieving this. One is by utilizing hyperbaric oxygenation. This technique has been shown to reduce the incidence of ventricular fibrillation following experimental acute myocardial infarction (11–15), to lessen the area of infarction following coronary ligation in dogs exposed to hyperbaric oxygenation as compared to those breathing room air (16), and to result in less of a rise of serum glutamic oxalacetic transaminase following experimental myocardial infarction in dogs exposed to hyperbaric oxygen than in those breathing room air (17). Our own experiments have shown reversal of post-infarction myocardial "excess lactate" production, and slight, though variable, improvement of the cardiac output during hyperbaric oxygenation (18). Coronary flow did not change despite increase of coronary vascular resistance. Aortic pressure increased significantly, attributable to a pronounced increment of systemic vascular resistance. It is possible that the apparently lessened lactate production attributable to hyperbaric oxygenation resulted from inhibition of glycolytic enzymes (19, 20), rather than increased oxygenation of ischemic tissue, so we are not certain from measurement of these parameters that the increased physically dissolved oxygen in the capillaries actually diffuses into the ischemic myocardium. However, the lessened susceptibility to arrhythmias and diminished areas of infarction associated with hyperbaric oxygenation suggest that increased myocardial oxygenation does, in fact, occur. There have been too few clinical trials to assess the advantages or hazards of hyperbaric oxygenation in humans with acute myocardial infarction. The many problems with the clinical use of this technique, however, will probably interdict its widespread employment in the future. These include its considerable initial and maintenance expenses, and the necessity for determining appropriate schedules to avoid the hazards of oxygen toxicity, pressurization, and decompression. These problems, of course, are not insurmountable, but it appears at present that reliable data establishing or disproving the efficacy of hyperbaric oxygenation are not sufficiently clear to warrant general use at this time. It is probable that in selected instances such as refractory arrhythmias, hyperbaric oxygenation may be useful as an adjunctive technique.

The other principle of achieving increased oxygenation of the acutely ischemic myocardium is that of raising the coronary flow. Probably the only way to achieve a substantial rise in coronary flow is to increase coronary perfusion pressure, that is, aortic pressure). There is solid rationale in attempting to increase coronary flow by this method in acute myocardial infarction with shock. Coronary perfusion pressure is a fundamental determinant of coronary flow, particularly when there is considerable hypotension. Elevation of a depressed coronary flow improves the function of both ventricles, thus improving the cardiac output, and lessens susceptibility to significant arrhythmias. Of course, by increasing coronary flow by elevating aortic pressure, the theoretic problem of increasing oxygen requirement of the ischemic myocardium immediately presents itself. In fact, advocates of the use of beta adrenergic drugs or alpha adrenergic blocking agents for the treatment of acute myocardial infarction with shock have stressed the desirability of reducing (or not elevating) arterial pressure, and hence myocardial oxygen requirement (21). However effective such regimens may be in the treatment of shock-like states resulting from conditions other than acute myocardial infarction, there is no credible evidence of beneficial results when they are used in acute myocardial infarction with shock, where the necessity for prompt increase in the coronary flow appears to be of more critical importance.

In experimental acute myocardial infarction with shock, mechanical increase of coronary flow achieved by increasing central aortic pressure by means of balloon obstruction of the abdominal aorta (Fig. 1) results in favorable hemodynamic and cardiac metabolic alterations despite concomitant increment of myocardial oxygen consumption (22). The degressed cardiac output rises (Fig. 2), and pretreatment post-infarction anaerobic metabolic al-

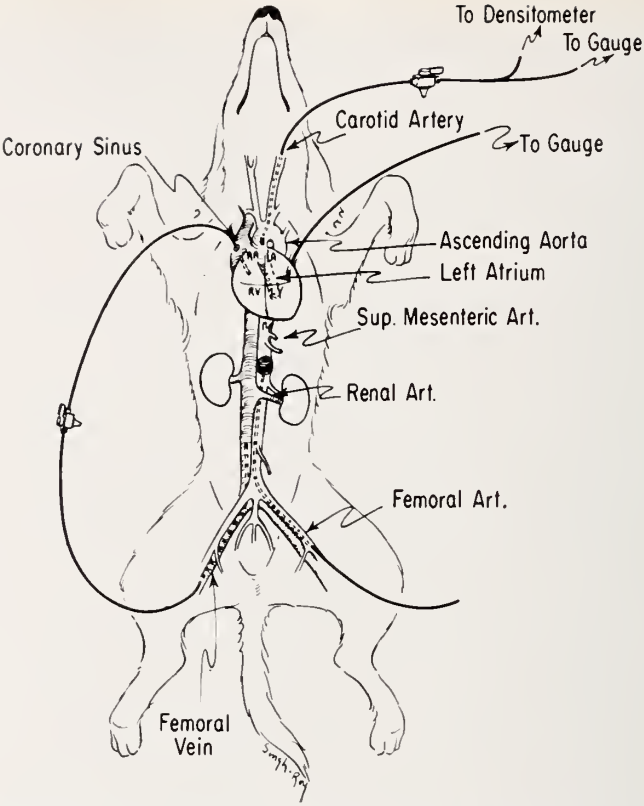

Fig. 1. Schematic representation of experimental arrangement. Circulatory compartmentalization is achieved by a balloon catheter inserted via a femoral artery into the abdominal aorta. Cannulation of the coronary sinus permits measurement of its flow (by gravity drainage or via flowmeter placed in the line), oxygen, lactate, and pyruvate content. Additional catheters permit similar simultaneous chemical determinations on arterial blood, measurements of left atrial and aortic pressures, and determination of cardiac output (22) (reprinted by permission of American Heart Association, Inc.).

terations are reversed when the coronary flow is increased. Therefore, the increment of ventricular oxygen requirement produced by the rise of central aortic pressure appears to be met adequately by the substantial increment of coronary flow resulting from a sharp rise of perfusion pressure from the depressed level of the shock state. Indeed, arterial-coronary sinus oxygen difference diminishes with the increased perfusion pressure, and there is a greater proportional increase in coronary flow than in perfusion pressure, probably resulting from maximal coronary dilatation secondary to myocardial ischemia.

These principles appear to be substantiated when one analyzes pharamcologic and circulatory support "therapy" in clinical and experimental acute myocardial infarction with shock. In our (23) own experiments, the purely beta adrenergic agent, isoproterenol, failed to produce a rise of coronary perfusion pressure or coronary flow in acute myocardial infarction with shock, and did not produce significant sustained improvement of cardiac output

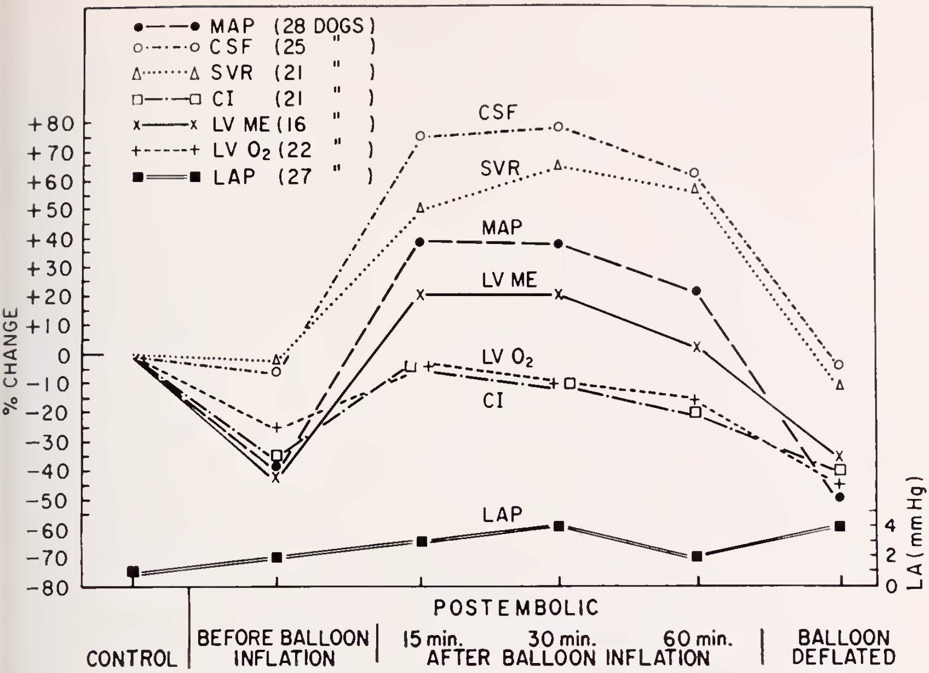

FIG. 2. Changes in hemodynamic measurements and O_2 consumption of the left ventricle produced by mechanical increase of systemic vascular resistance in experimental acute myocardial infarction induced by coronary embolization with plastic microspheres. These are paired determinations with each animal as its own control, expressed as average percentage variations from the control values obtained after anesthesia prior to the production of myocardial infarction. Increments of cardiac output, stroke volume, coronary flow, left ventricular oxygen consumption, and left ventricular "mechanical efficiency" produced by balloon aortic obstruction in the post-infarction state were significant at less than the 0.05 level of probability. Significant diminution ($p < 0.05$) of arterial-coronary sinus oxygen difference and reversal of ventricular excess lactate production were also produced, suggesting improvement of myocardial oxygenation. LV ME = left ventricular mechanical efficiency (%); LV O_2 = left ventricular oxygen consumption (ml/100gLV/minute); and LAP = left atrial mean pressure (mm Hg) (22) (reprinted by permission of American Heart Association, Inc.).

or post-infarction anaerobic ventricular metabolic alteration. Similar failure to improve hemodynamic alterations in clinical acute myocardial infarction with shock has also been reported (24), but the effects of this drug are controversial. Because it increases the oxygen requirement of the myocardium (by increasing the rate of rise of left ventricular pressure) beyond its ability to increase coronary perfusion pressure and flow, isoproterenol may be associated with increased post-infarction myocardial excess lactate production. In contrast, the alpha-and-beta-adrenergic agent, norepinephrine, which produces significant increment of aortic pressure and coronary flow in acute myocardial infarction with shock, results in improvement of the low cardiac output and reversal of myocardial anaerobic metabolic changes in about two-thirds of the animals studied (25). These apparently beneficial hemodynamic and cardiac metabolic alterations occur despite a substantial increment of left ventricular pressure work and increments of left ventricular myocardial oxygen consumption equal to those produced by isoproterenol.

Analysis of experimental data obtained with the use of mechanical circulatory assistance when severe hypotension is the major hemodynamic abnormality in myocardial infarction with shock, also indicates the desirability of substantially raising coronary perfusion pressure. Venoarterial shunting, which may be helpful in treating acute volume overloading of the right ventricle, as in sizeable pulmonary embolism, is generally ineffective in shock due to acute myocardial infarction (26). In our experience, counterpulsation of aortic blood, even when performed in the central aorta, is only slightly and inconsistently effective in reversing the hemodynamic and cardiac metabolic abnormalities in acute myocardial infarction with shock (8). We believe this is related to the fact that this technique is incapable of achieving substantial elevation of the depressed coronary perfusion pressure when hypotension is marked, even though it effectively "reverses" the aortic pressure pulse, and thereby results in some diminution of myocardial oxygen consumption. Our results indicate that the depressed coronary flow of myocardial infarction with shock is not increased by central aortic counterpulsation of blood, despite some rise of the diastolic pressure, probably because the degree of rise is inadequate for this purpose, and possibly also because of concomitant peripheral coronary vascular adjustments. However, when the same technique of central aortic counterpulsation is accompanied by circulatory "compartmentalization" (26), achieved by balloon obstruction of the lower abdominal aorta, anaerobic myocardial metabolic alterations are reversed and there are substantial elevations of cardiac output, coronary perfusion pressure, and coronary flow. These results are achieved at minimal oxygen consumption increment because of the simultaneous effects of counterpulsation (8).

One currently utilized technique which apparently applies these principles is counterpulsation achieved by means of phasic deflations and inflations of a balloon placed in the upper thoracic aorta. While this technique was advocated originally as a convenient means of counterpulsation which would not require pumping of blood (27–29), we believe its efficacy rests on principles other than those obtained by counterpulsation of blood. As stated previously, when there is significant hypotension following acute myocardial infarction, central aortic counterpulsation effects insufficient changes of the aortic pressure phases to produce substantial increment of mean coronary flow. When an aortic balloon is inflated and deflated to achieve the purposes of counterpulsation, there is a resultant greater increment of coronary perfusion pressure and flow than exists with counterpulsation of blood alone. This is attributable to circulatory compartmentalization produced by balloon aortic obstruction, the concepts and the beneficial effects of which our own group originally developed and stressed (26, 30). Thus, aortic balloon phasic inflation and deflation achieves the advantages of both counterpulsation and circulatory compartmentalization, both of which may be necessary in extreme hypotension associated with acute myocardial infarction. However, in extreme hypotension, even balloon aortic counterpulsation may be ineffective in relieving the observed hemodynamic and cardiac metabolic abnormalities. In such instances, beneficial effects may be obtained by further circulatory compartmentalization, produced by inflating an additional balloon located distal to the main pumping balloon (31), or placement of a distal "valve" restricting cardiac stroke ejection to the proximal aortic segment (32).

Clinical Application

There has been very limited clinical application of circulatory support systems in non-surgical cardiac conditions. This is probably attributable to both organizational and medical factors. In our own hospital, the total number of potentially eligible patients developing the shock syndrome is estimated at about 50 per year. Of these, a minority (about 20 percent) will respond adequately to available medical therapy. An additional number expire before appropriate circulatory support therapy can be instituted, and still others have significant complicating conditions which require other means of treatment, such as tachy-arrhythmias or brady-arrhythmias necessitating pacemaker insertion. Therefore, it is difficult for a single, large-sized institution to accumulate, in a relatively short time, large numbers of patients appropriate for study of these techniques. Organizationally, the optimal application of these methods requires extremely close medical-surgical-engineering cooperation and expertise, which is not available in many institutions. Nevertheless, some data involving human subjects are available which can be analyzed briefly here.

Techniques of left heart bypass, generally involving blind, transjugular transseptal cannulation of the left atrium, have been applied to a small number of patients (33, 34). The efficacy of this technique is difficult to assess because of limited data in the patients, and because it has been applied in a variety of conditions other than acute myocardial infarction. Its application is really for volume overloading of the left ventricle, with or without acute myocardial infarction, and with or without shock. Transjugular transseptal cannulation is a difficult technique which has not had widespread application, in part due to this assessment of its feasibility. A more satisfactory approach to achieve the same purpose may be direct left ventricular drainage through a large-bore catheter inserted via a peripheral artery into the left ventricle. An appropriately designed catheter has been found to support the circulation adequately, even during experimental ventricular fibrillation (35). Our current research efforts are directed to utilizing such a technique with an appropriately phased counterpulsation apparatus, which may be combined with aortic balloon inflation to provide for both ventricular drainage and maximum coronary flow during diastole. There has been no clinical application of direct left ventricular drainage, however.

Another approach, which has been used in surgical patients, but not in acute myocardial infarction, is that of direct cannulation of the left atrium with left atrial-aortic shunting through a pump. Although traditionally open-chest techniques have been avoided in acute myocardial infarction, it is entirely possible and likely that effective reduction of acute volume overloading of the left ventricle may be achieved by this means. Acceptance of the putative risks of the procedure may be warranted, in view of the grave prognosis, when there is insufficient response to appropriate medical therapy.

Counterpulsation of blood (arterio-arterial) (36, 37) has also had limited clinical application, with insufficient data accumulated as yet to fully assess the value of the procedure. The technique which has had widest clinical use to date is that of intra-aortic phased balloon pumping. The bulk of this experience has been accumulated by one group, utilizing helium as the inflating gas (38). About 30 patients have been subjected to this procedure at the time of this writing (July 1969), all of whom are reported to have been in cardiogenic shock unresponsive to conventional medical management. The authors report at least temporary relief of "shock" in almost all patients, with ultimate survival in about 40 percent, the prognosis being distinctly superior in patients who develop the shock syndrome soon after the onset of their infarction, as compared to those developing shock a few days after the onset of the infarction. In the latter group, relatively little success has been obtained.

This procedure appeared rational, but in severe hypotension, or with significant volume overloading of the left ventricle, additional modifications will probably be necessary, as discussed previously.

Two considerations emerge as a result of evaluation of the limited clinical experience with this technique. While it is probable that the lives of some of the patients refractory to usual forms of therapy for acute myocardial infarction have been saved, it should be realized that, as with any investigative technique, intensive care was applied, particularly vigorous fluid therapy. The role of such measures in the ultimate survival of the patients will have to be assessed in the future, preferably by random selection of patients. One disturbing feature is a high incidence of reported ventricular rupture in the patients not surviving (5 of approximately 24 deaths), although the precise figures are not available at this time. This is a much higher incidence of ventricular rupture than generally reported during the natural course of acute myocardial infarction. While it is possible that circulatory assistance enabled the patients to live longer than they would have otherwise, thus permitting more extensive infarction to develop with resultant cardiac rupture, it is also possible that the ruptures may be attributable to the technique itself. This complication has not appeared in experimental investigations, and its mechanism is not clear. One might postulate, to explain this, either ventricular "overloading" due to improper phasing of the balloon, or aortic regurgitation related to high diastolic pressures. Neither of these has been established clinically or experimentally, and at this point it seems reasonable to conclude that application of phased balloon inflation in the aorta may be a useful therapeutic modality in selected patients with acute myocardial infarction with shock.

Future Trends

The field of circulatory assistance in acute myocardial infarction with shock is in its earliest phases. One task is to determine sequential alterations of hemodynamics from the earliest phases of the syndrome. This will enable more precise definition of the prognosis, and earlier application of appropriate support techniques at a phase of the illness prior to the development of irreversible circulatory and metabolic deterioration. In the future, this may be aided by computer detection of subtle trends of reduction of cardiac output, arterial pressure, or more simply measured clinical or laboratory detected parameters. If the prognosis of an early trend can be accurately determined, a mechanical or pharmacologic therapeutic technique may be applied successfully relatively early in the course, whereas our present criteria for therapy are probably reflections of relatively long-standing and profound circulatory and cardiac deterioration.

Another important task is to separate the broad clinical syndrome of shock following acute myocardial infarction into homogeneous hemodynamic subgroupings. Undoubtedly, different techniques will have to be applied for different hemodynamic abnormalities. A technique applicable when severe hypotension is the major abnormality, such as balloon inflation of the aorta, may be ineffective when volume overloading of the left ventricle is the principal hemodynamic abnormality, where left ventricular bypass or direct left ventricular drainage appear to be indicated. Hyperbaric oxygenation and/or moderate hypothermia may be useful adjunctive techniques in certain instances in which there is severe circulatory deterioration. It can be predicted with reasonable confidence that we will see more intensive efforts to apply mechanical circulatory support in the future, with its attendant close medical-surgical-bioengineering cooperation. It is the by-product of such cooperative efforts that may provide further advances in many additional areas of cardiology.

Summary

The rationale, desirable physiological objectives, and current state of circulatory support devices in acute myocardial infarction are reviewed. Analysis of available data indicates that there are a variety of hemodynamic patterns occurring in different individuals, with the common clinical appearance of shock following acute myocardial infarction. On the basis of experiments and clinical data, an attempt is made to determine physiologic objectives to be met by systems of circulatory support designed to relieve the observed hemodynamic abnormalities. Because shock may appear in different hemodynamic "settings," different principles and techniques are necessary in the treatment of patients with this syndrome who do not respond to conventional medical therapy.

The patient with hypotension as the dominant hemodynamic abnormality may require prompt and sustained mechanical elevation of aortic pressure, thus increasing a previously diminished coronary flow to the acutely ischemic ventricle. Experimental evidence is presented which indicates that mechanical increase of central aortic pressure in acute myocardial infarction with shock produces beneficial hemodynamic effects and reversal of anaerobic ventricular metabolic alterations, despite the fact that left ventricular "work" and oxygen requirement are thereby increased. The increase of coronary flow produced by

increments of perfusion pressure appears adequate to meet the increased oxygen needs of the myocardium. Techniques which have as their sole purpose reduction of left ventricular work are less effective in relief of shock following acute myocardial infarction, although they may be helpful when combined with other techniques which increase coronary perfusion pressure and flow. When acute volume overloading of the left ventricle is the principal hemodynamic characteristic, techniques of left ventricular bypass or left ventricular "drainage" are indicated. It is emphasized that reduction of the oxygen requirement of the left ventricle may require very large volumes of shunt flow, but that effective left atrial pressure reduction may be produced by bypassing considerably less blood, about 25 percent of the total left ventricular volume.

The available techniques of circulatory support are outlined. The importance of circulatory compartmentalization in raising coronary perfusion pressure and flow from shock levels is indicated. Preliminary clinical data are analyzed which demonstrate some improvement and survivals in refractory patients with acute myocardial infarction with shock treated with phasic inflation and deflation of a balloon catheter placed in the descending aorta. Some of the uncertainties in interpretation of the data, and the relatively high incidence of ventricular rupture which appeared in these patients are discussed.

Based on presently available knowledge and projected future research trends, it is felt that future efforts will be devoted to more precise analysis of the hemodynamic, particularly intracardiac, alterations occurring in myocardial infarction with shock, with resultant separation into more precise subgroupings. This should permit more accurate determinations of prognostic trends in the patient's course. One or a combination of appropriate mechanical circulatory aids may then be applied promptly, before irreversible circulatory deterioration.

Acknowledgments

The investigations on which these conclusions are based were carried out over a period of several years. These were performed with the important aid of many Research Fellows of the Division of Cardiology of the Department of Medicine, all of whom I cannot cite here. I do wish to acknowledge the invaluable and more prolonged assistance of Doctors Howard Kline, Anthony Marano, Allen Unger and, more recently, Stephen Novick.

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Current Status of Myocardial Mechanical-Energetic Relationships*

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During the last few decades much effort has been expended in the basic sciences. One of the areas to most benefit from this has been the field of clinical cardiology, where increased information regarding the relationships of fundamental phenomena has been applicable to clinical states. Appreciation of mechanical and energetic principles has led to a greater understanding of the metabolic burdens imposed in states that are associated with cardiac dilatation, valvular, and ischemic heart disease.

There are many energy consuming processes in the heart. Among these are the basal metabolic requirements of the tissue for normal survival and renewal, which probably accounts for less than 20 percent of the total energy use by the working heart (1). Energy is consumed for ion transport, which serves to maintain transmembrane gradients and confer the characteristics of automaticity and excitability upon the heart. These processes probably require less than 1 percent of the total cardiac energy expenditure (2). Energy is utilized for transport and intracellular binding of calcium, which is involved in the excitation-contraction-relaxation cycle (3). The major energy requirement is for the contractile process, and is thought to be involved in the interaction of actin and myosin (4).

The purpose of this paper is to outline the relationship between various measurements of myocardial energy utilization and production and myocardial mechanics, illustrating how knowledge of these relationships affords a better understanding of clinical events.

Reports that deal with either myocardial contraction or energy utilization frequently employ their own set of terms to describe the events under study. The following section is intended to define terms as they will be used in this review.

The basic unit of contraction in cardiac muscle, as well as in skeletal muscle, is the sarcomere. Certain models have been proposed in order to describe the external manifestation of the internal contractile events in the sarcomere. Although recent studies have suggested modification of the classic model (5), under most circumstances cardiac muscle can be considered to be a three component system. The contractile element (CE) and an elastic component in series with it, the series elastic element (SE), are considered to be representative of components within the sarcomere proper. The relationships of the con-

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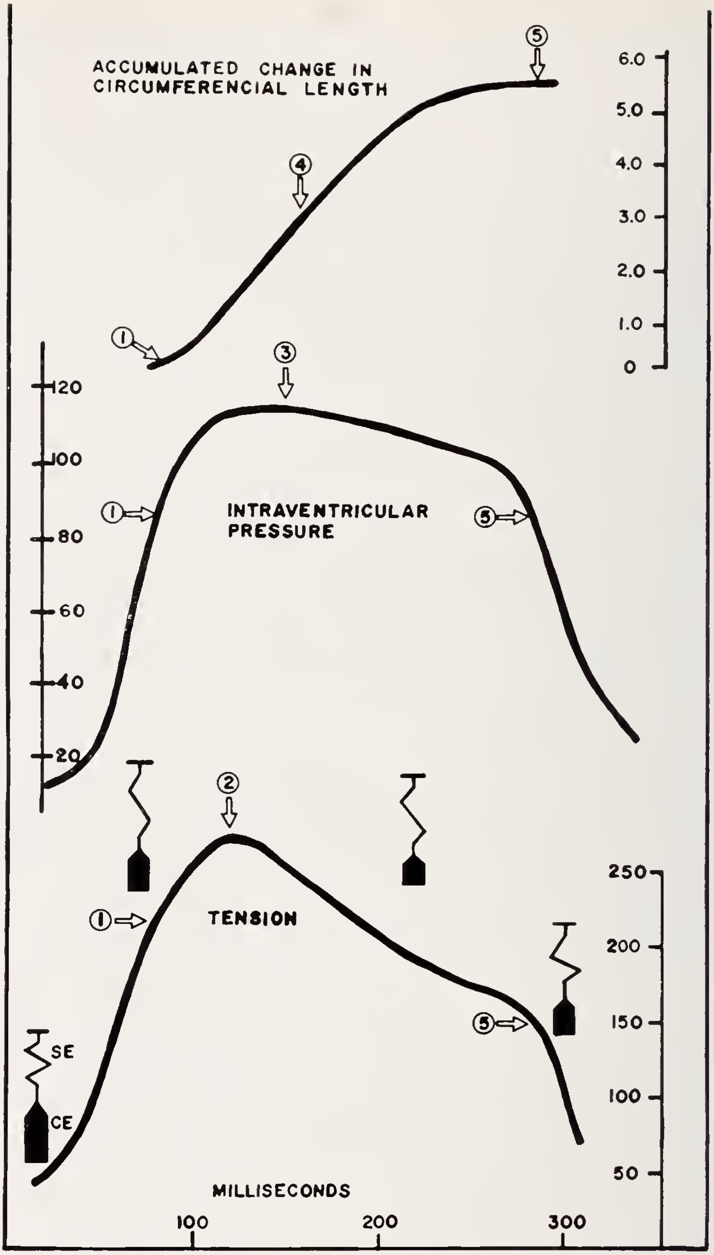

FIG. 1. This figure is a schematic representation of the relationships between circumferential shortening, intraventricular pressure, and wall tension during ventricular systole, as derived from the data of Gault, Ross, and Braunwald (6). The abscissa for shortening represents accumulated change in centimeters, for intraventricular pressure mm Hg, and for tension g/cm^2. Proposed changes in the contractile elements and series elastic element during the phases of the cardiac cycle are shown above the tension plot. The solid portion of these diagrams represents the contractile element (CE) and the folded line represents the series elastic element (SE). At zero time, before contraction begins, the CE and SE are at initial length. At ①, the time of semilunar valve opening, the overall length of the CE plus the

tractile element, the series elastic element, development of tension, the external pressure, and shortening during a cardiac cycle are schematically illustrated in Figure 1. A parallel elastic unit is thought to reside within the connective tissue. The last of these is manifested only at rest, and has no real effect on the developed tension or shortening that occur during contraction. Its significance may be of importance in dilated hearts and hearts working under markedly increased loads, but this possibility has not been satisfactorily evaluated. The ability to dismiss the parallel elastic element in such a fashion points up a distinct difference between cardiac and skeletal muscle. Skeletal muscle in the body is at or near the length which will result in the maximal development of shortening, and /or tension. This is in contrast to cardiac muscle, which in a basal state in a healthy individual is shorter than the optimal length for work or tension development.

Since the ultimate object of many energetic studies is to quantitate the activity of the heart in engineering terms, the idea of work rather than tension development is more appealing. Thus, a cardiac contraction may be thought of in terms of the work of the contractile element (CEW), rather than in terms of the more common but more complex manner in which a cardiac contraction has been classically presented. The latter speaks of isometric contractions in which tension is developed at a constant muscle length. Isotonic contractions are contractions which produce shortening at a constant tension. A normal cardiac contraction is a combination of the two. Often called auxotonic or afterloaded contraction, as shown in Figure 1, the initial phase of contraction prior to valve opening is isovolumic, if not actually isometric. During this phase tension rises rapidly, and the CE shortens and the SE lengthens, but no external cardiac work is performed. During the first one-third of ejection the tension rises at a slower rate (6), and external shortening is due to continued contractile element shortening. The propulsion of blood against peripheral vascular resistance during ejection is the external work performed by the heart.

In contrast to the variety of terms usually applied to the contraction of the whole heart, consideration of the heart in terms of the contractile element merely states that the CE does internal work by shortening until it has stretched the SE spring equal to the load, and it then does external work by further shortening until the activity of the CE declines and relaxation begins. A major advantage of thinking of contraction in this manner is that all activity is quantitated in terms of work.

The second advantage of considering contractions in these terms is that experimental data obtained with different preparations should be more easily comparable. While in theory this is an acceptable stance, one problem is that

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SE are the same, but the CE has shortened and the SE has lengthened. External shortening then begins. Tension rises to a peak at ②, earlier than peak pressure, which occurs at ③, and peak rate of shortening, which occurs at ④. CE and external shortening continue throughout ejection with little change in the SE. By ⑤, the time of semilunar valve closure, relaxation of the CE and SE have begun. In diseased ventricles, points ② ③, and ④ maintain their temporal relationships, but occur later in the ejection phase. With increases in contractility, these points occur earlier.

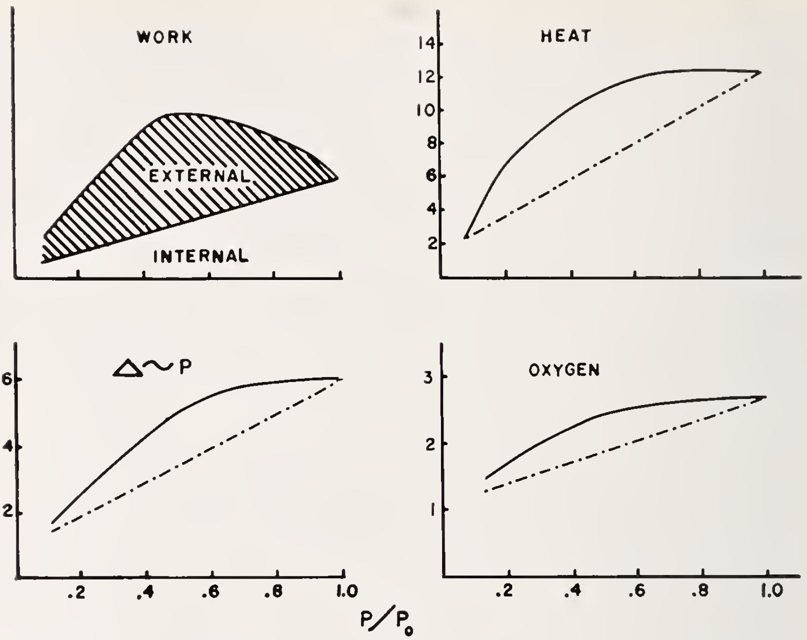

FIG. 2. Cardiac energetics in papillary muscles as expressed by work, heat production, change in high energy phosphate, and oxygen consumption. The curves represent data derived from the studies by Gibbs, Mommaerts, and Ricchiuti (10), Pool et al (9), McDonald (41), and Coleman (8). Except in the work panel, the dashed lines represent the energy for internal (isometric) work, and the solid lines represent the energy for total work during shortening against a load (isotonic work). The abscissa for high energy phosphate ($\Delta \sim P$) is in μmoles/g-cm. for heat is m cal/g, and for oxygen is ml/g. The ordinate represents the ratio of the actual load, P, to that load against which the muscle cannot shorten, P_0. P_0 is the isometric load. The work panel, upper left, demonstrates the linear relation between load and internal contractile element work as the load is increased. This is the work expended in stretching the SE before shortening can occur. As shortening occurs, added CEW is performed, and the total work is non-linear, but reaches a maximum at approximately half the P_0. As the load is increased beyond that point, the total work of the contracting muscle declines slightly, although internal CEW continues to increase. The relationships of internal (isometric work) and external work (shortening against a load) are reflected in each of the energy panels. The area between the line for the internal work and the curve for the external work represents the energy consumed by lifting the given load and represents the Fenn effect.

the concept is too recent to have been properly considered in most past studies of the relationship between energy utilization and mechanical activity. When contraction is considered in terms of CEW, it becomes clear that cardiac muscle, like skeletal muscle, shows the phenomenon known as the Fenn effect (7). First described by Fenn in skeletal muscle in 1923, and recently demonstrated in cardiac muscle (8–10), the Fenn effect states that when a muscle does external work, energy is consumed in excess of that predicted by tension requirements. Its presence is clear; its cause is not. The relationships of these two forms of work to heat production, oxygen consumption, and high energy phosphate utilization are shown in Figure 2.

A second problem in considering cardiac activity in terms of contractile element work is the dearth of studies in the intact hearts which use this concept as a base. This is in part due to the difficulty of calculating internal work or tension in the intact heart. For continuity, we will try when possible to relate various studies to contractile element terms so that a common framework is available.

A third area to consider is the difference between skeletal and cardiac muscle in regard to the phenomenon of inotropy, or contractility. Changing the inotropic state is a fundamental property that determines the way the heart responds to calcium and certain pharmacologic agents, such as digitalis glycosides and hormones, such as glucagon, or the catecholamines. The terms inotropy and contractility have been defined in a wide variety of ways. In terms of contractile element function, positive inotropy is a change in the muscle characteristics, which result in a faster rate of contractile element work for the same sarcomere length, with no change in the series elastic characteristics (11). This is often expressed in terms of the maximum velocity (V max) that a muscle in such an inotropic state would obtain if it contracted with zero load. The ability to do more contractile element work from the same initial length usually occurs, but need not be present.

Heat Production and Myocardial Function

Just as the definition of contractile events in terms of contractile element work should give a simpler definition of cardiac effort, quantitation of cardiac energy utilization in terms of a common energy term would simplify the understanding of energetics. This can be done by the measurement of myocardial heat production. Since heat production is a measure of the total energy turnover, it would appear that measurement of heat and CEW is an index of the efficiency of the contractile process. However, in the absence of a measure of the chemical input into the system, it is only a relative index of efficiency. No studies of myocardial contraction and energy utilization reported up until the present time have measured true efficiency. The logistic problems in executing simultaneous heat, biochemical, and mechanical measurements are formidable.

Most of the heat production studies have been performed in skeletal muscle, working at low temperatures. The results, while directionally informative, cannot be strictly applied to cardiac muscle. Beginning with the papers of Heidenhain (12) in 1864 and Hill (13) in 1911, it has been demonstrated on numerous occasions that in an isometric contraction the force developed and the energy liberated are dependent on muscle length. Furthermore, the development of the force has a proportional expenditure of energy. In skeletal muscle, the ratio Pl_0/H, where P is the force developed in the isometric twitch, H the heat produced, and l_0 the standard resting length, is a constant (14, 15). In skeletal muscle, it is approximately 11 g-cm/cal. The number would be lower in cardiac muscle because of increased elasticity, but as yet it has not been measured.

What happens in skeletal muscle when shortening is allowed to occur is not as clear. In fact, this has been the basis of controversy since 1923, when Fenn first described the additional heat evolved when a muscle was allowed to shorten against a load, as noted above. In 1938, Hill (16) presented data which he interpreted as showing that this heat had two components: (1) heat due to the load lifted, that is the mechanical work; and (2) heat due to shortening. In 1949, he published further data, which he interpreted as showing that in a single twitch shortening heat was produced independent of the load (17). However, the more recent work of Carlson and Siger (18) with phosphocreatine measurements, and of Carlson, Hardy, and Wilkie (15, 19) with simultaneous measurements of heat, work, and phosphocreatine breakdown has called these interpretations into question. They found that the heat produced and the phosphocreatine hydrolyzed correlated well with the load lifted, but they could not demonstrate a separate shortening component. In addition to heat released for contraction, a small quantity of heat liberation has been reported for activation alone (10, 20).

One area of interest in cardiac muscle is the effect of velocity on energy turnover. There are no comparable studies in skeletal muscle. Changes in contractility can be represented by changes in the extrapolated maximum velocity (V max) of the contractile element. This is an area of great interest in the heart, where it can be illustrated that administration of norepinephrine, calcium, glucagon, or digitalis glycosides alter contractility. While caffeine does much the same in skeletal muscle, we are unaware of any studies which have investigated this in terms of heat production.

Measurement of myocardial heat production, while necessary for a knowledge of cardiac efficiency, has only been successful in recent years. Earlier work by Fisher (21) in 1925, and Neill et al (22) in 1961 concluded that reliable measurements were not available because of the variable heat gradient obtained and irreproducibility of the data. Such irreproducibility also plagued the studies of Afonso and associates (23), who attempted to avoid the common problems of measurement by using a thermal dilution method. In 1967, Gibbs, Mommaerts, and Ricchiuti (10) reported the first reproducible measurement of myocardial heat production. Using isolated rabbit papillary muscles at 20°C, they showed that there is a linear relationship between actively developed tension and heat production. In addition, they demonstrated the production of heat over and above the heat due to tension when a load was lifted by the muscle. Thus, they felt that the Fenn effect had been demonstrated in cardiac muscle. Gibbs (24), in a later report, has shown that the addition of epinephrine to the system results in a greater amount of heat liberation at the same level of tension development, suggesting that changes in contractility may cause additional heat production. More recently, one of us (RHM) has been able to develop an isolated perfused heart preparation in which total myocardial heat production at 37°C can be studied under steady state conditions. A linear relationship between the tension developed and the heat produced is seen. The relationship is altered by the infusion of norepinephrine, demonstrating the influence of inotropic change. The work on cardiac heat production is incomplete and needs further exploration before the knowledge of cardiac energetics approaches that of skeletal muscle.

Oxygen Consumption and Cardiac Function

Since the measurement of myocardial heat production is still in its infancy, other measures of energy turnover must be used. The one most often used has been myocardial oxygen consumption. The number of reports is vast and rather than analyze each in detail, we shall try to present a historical overview and point out those papers which seem to us to permit greater accuracy in relating oxygen consumption to mechanics, or to conceptually contribute to our understanding.

Nearly 60 years ago, Rohde and Ogawa (25) demonstrated in an isovolumically contracting heart that the oxygen consumption increased as the pressure in the ventricle increased. In 1914, Evans and Matsuoka (26), using a heart-lung preparation, performed their classic experiments which demonstrated that "pressure work" consumed more oxygen than "flow work." The rediscovery of this finding has occurred several times each decade since then. They state: "We may assume that the diastolic volume of the heart is not increased so much in order to produce a given increment of work by increase of output as to produce the same increment by increase in arterial pressure. This brings our observations into line with those of A.V. Hill on skeletal muscle. In both cases there is a relation between the tension set up on contraction and the metabolism of the contractile tissue. The case of a heart working against high pressure is somewhat like that of muscle contracting against a strong spring, i.e. it more nearly approaches isometric conditions, while the ventricle with a high systolic output is comparable to a muscle executing considerable shortening against a weak spring, i.e. it is more nearly isotonic. The tension developed is greater in the former case. The tension is more completely converted to work in the latter case."

They also pointed out that it was the magnitude of tension development, rather than its maintenance, which seemed to have the most influence on oxygen consumption.

Around 1926, Starling and Visscher (27) and Hemmingway and Fey (28) reported correlations between diastolic fiber length and myocardial oxygen consumption. While work since that time has shown the correlation to be poor, at the time it was greeted with a certain amount of enthusiasm since it suggested that there was a chemical energetic basis for Starling's law of the heart. Further understanding of various interrelationships had to wait until the late 1950's and the present decade. In 1958, Sarnoff et al (29) suggested that the primary determinant of oxygen consumption was the total tension developed by the myocardium, as indicated by the area beneath the systolic pressure curve. They called this the tension-time index (TTI). They further recognized that this really was an index of tension development rather than an actual measure of tension, and that as ventricular dimensions changed, the relationship between the tension-time index and oxygen consumption would change, since a larger heart requires greater development of tension for the same amount of pressure development. Not only did this paper begin the modern era of measurement of myocardial oxygen consumption, but it also evoked enough controversy that it might well be considered a fuse for the explosion of data that followed.

A number of authors were unsatisfied with external pressure measurements as an index of tension development. The effect of changing ventricular size on the tension required to develop a given pressure was calculated to increase significantly with cardiac dilatation (30). Rodbard, Williams, and Williams (31) attempted to resolve the problem of tension and ventricular volume by inclusion of stroke volume as a measure of ventricular volume. Although this works well at small ventricular volumes, since the end systolic volume is ignored, an increasing error is introduced as end-systolic volume increases. Utilizing an isovolumetric balloon filled preparation, Monroe and French (32) demonstrated that peak developed tension and an integral of systolic pressure in time correlated equally well with oxygen consumption. In a later paper, Monroe (33) demonstrated that in such a preparation, at least 91 percent of the oxygen cost of contraction has been determined by the time peak aortic pressure has been obtained, suggesting that peak tension might be of more importance than the duration of tension maintenance in determining O_2 consumption.

In 1966, McDonald, Taylor, and Cingolani (34) attempted to measure developed tension in an intact heart by calculation of the tension in the wall at 20 m sec intervals throughout the cardiac cycle. While the correlation of changes in oxygen consumption with changes in tension was good, it appears that they underestimated wall tension by 10 to 15 percent. Furthermore, they were unable to calculate the influence of shortening changes. Later work by Taylor et al (35) added information confirming an effect of shortening on oxygen consumption. Graham et al (36) added the effect of the contractile state as a determinant of myocardial oxygen consumption. Thus, basal oxygen consumption, tension development, extent of fiber shortening or external work, and the contractile state had all been demonstrated as contributing to the total oxygen consumption of a heart. An important conceptual advance in recent years has been the attempt by Britman and Levine (37) to quantitate myocardial oxygen consumption in terms of contractile element work. Although they obtained a good correlation in a limited series, this correlation has not held up in other laboratories. Rather than dismiss this as an interesting theoretical exercise, it seems worthwhile to examine the causes of the discrepancies. They extrapolated their spring constant from the data of Sonnenblick on isolated cat papillary muscle. Most recent data show that the elasticity of the intact ventricle, and indeed isolated papillary muscle, is about half the previous estimate (38). Reexamination or repetition of earlier experiments would seem to be indicated in the light of that error.

One of the problems of studying the various interrelationships in intact hearts has been the problem of how to measure changing ventricular geometry, volume, and fiber shortening. In an attempt to simplify and view the various problems individually, a number of investigators have attempted to use isolated tissue. The papillary muscles, especially from the right side of a small mammalian heart (cat or rabbit), are ideally suited since they are generally thin and oxygen diffusion limitations are a minor consideration. Secondly, their geometry is essentially a series of parallel contractile elements, in contrast to ventricular fibers, which at any section may be as much as 120° out of phase in their orientation at any part of the ventricular wall.

Lee (39) and Whalen (40) deserve the credit for first recognizing and investigating the potential of this approach. Lee demonstrated a relationship between isometric tension developed and oxygen consumed. When Whalen studied muscles that were shortening externally, his system was insensitive to the oxygen changes occurring, and he was led to believe that energy utilization was quantal in nature and not of the type seen in skeletal muscle. In 1966, McDonald (41) was able to demonstrate that changes in developed tension and contractile element work were paralleled by changes in oxygen consumption of the isolated papillary muscle. This investigation included studies on muscles that were contracting isometrically or shortening while lifting a load. Utilizing the same apparatus, Coleman (8) extended these findings and demonstrated alterations in oxygen consumption related to shortening and external work, as well as to contractility (42). In his data, as well as much of that derived from whole heart studies in which the effect of shortening was considered, the Fenn effect could be demonstrated. Thus, whether measured by heat, oxygen, or high energy phosphate (vida infra) utilization, the effect of external load and shortening could be shown to cause energy turnover above and beyond that predicted from internal work. These relationships are shown in Figure 2.

High Energy Phosphate Compounds and Myocardial Function

As with heat, most of the investigations relating high energy metabolism to muscle function have been performed on skeletal muscle. Myocardial tissue has only been studied in this regard in recent years.

In 1907, Fletcher and Hopkins (43) reported that in the absence of oxygen, muscle contraction was accompanied by lactic acid production, suggesting that a complex process was involved in converting chemical to mechanical energy. Subsequent experiments by Parnas and Wagner (44), Meyerhof (45), and Hill (46) demonstrated that during anaerobic contraction, glycogen was converted to lactic acid in quantities that could account for the energy ex- and Hill (46) demonstrated that during anaerobic contraction glycogen was derived from glycogen. In 1930, Lundsgaard (47) disproved this theory. He used iodoacetate to block the glycolytic production of lactic acid in muscle, and found that contraction could still occur aerobically. Furthermore, it was observed that when iodoacetate treated muscles contracted anaerobically, creatine phosphate (CP) was broken down to creatine (C) and inorganic phosphate (Pi). It was later shown by Lohmann (48) that adenosinediphosphate (ADP) was an obligatory co-factor for this reaction. Thus, the theory was developed that ATP was the direct donor of energy for muscular contraction, and CP restored ATP by rephosphorylating ADP according to the reactions:

$$\text{ATP} \xrightarrow{\text{“ATPase”}} \text{ADP} + \text{Pi} \qquad \text{I}$$

$$\text{CP} + \text{ADP} \xrightarrow{\text{CPK}} \text{ATP} + \text{C} \qquad \text{II}$$

This theory was furthered by the observation that the contractile protein myosin had the enzymatic property of hydrolyzing ATP, and therefore served as the “ATPase” for reaction I (49), and also by the finding that ATP reacts directly with purified actomyosin systems (50, 51). To support the reservoir role of CP, Carlson and Siger (18) demonstrated that when ATP production was blocked, all of the energy for muscle contraction could be accounted for by CP breakdown. Later, Cain, Infante, and Davies (52) related CP utilization directly to the contractile phase of the contraction-relaxation cycle.

Even though there was much evidence for the sequence described by reactions I and II, it was not until 1962 that ATP was identified as the direct energy source for contraction of the intact muscle. Cain, Infante, and Davies (53, 54) used the compound 1-fluoro-2,4-dinitrobenzene (FDNB) to block the enzyme creatine phosphoryltransferase (or creatine phosphokinase (CPK)), thus preventing the rephosphorylation of ADP by CP, and blocking reaction II. They were able to demonstrate that without the availability of CP, ATP was broken down during each contraction. Since ATP was the only energy source, these experiments proved the direct role of this compound in muscle contraction.

In the experiments with FDNB, Cain and Davies (53) found the myokinase reaction to be highly active:

$$2\ \text{ADP} \xrightarrow{\text{myokinase}} \text{ATP} + \text{AMP} \qquad \text{III}$$

However, myokinase appears to be activated only when ATP declines during contraction (55). In cardiac muscle, marked changes in ATP do not normally occur. Therefore, the myokinase reaction would probably serve mainly as a reserve mechanism in the heart.

Attempts have been made to relate the phases of ATP breakdown during muscle contraction with the timing of heat production. Carlson et al (19), and Mommaerts, Seraydarian, and Marechal (56), studying CP breakdown, were able to derive chemical expressions related to the energy of activation and work performed. Mommaerts and Wallner (57) could not relate any ATP utilization for relaxation. Davies et al (54, 58) confirmed that the majority of ATP breakdown could be accounted for by contraction. An additional quantity of chemical energy was broken down during the relaxation phase and was thought to represent the energy of activation. However, this could account for only a small portion of the total heat of activation described by Hill (59). These authors postulated that this energy was utilized for the transport of calcium into the sarcoplasmic reticulum. Carlson et al (19), Mommaerts et al

(56), and Davies, Kushmerick, and Larson (58) could not identify any breakdown of high energy phosphate specifically due to shortening, and Davies et al postulated that heat of shortening may be due to the conversion of the H meromyosin from a random coiled form to a helical form, thus effecting sarcomere shortening through the mechanism of hydrogen bonding.

Direct linear relations between high energy phosphate utilization and both internal work and external work have been described in skeletal muscle (19, 60). The greatest tension and ATP expenditure occur when a muscle is contracted at its normal resting length. As the initial muscle length is either increased or decreased, the efficiency of converting chemical to mechanical energy during isometric contraction decreases (61), illustrating that in an isometric contraction, the force developed and the energy consumed are dependent on muscle length. This is analogous to the heat data discussed previously (13).

The investigations regarding high energy phosphates cited up to this point have all involved amphibian skeletal muscle studied at low temperatures. Studies in cardiac muscle have not been as extensive, but indicate a qualitative similarity with skeletal muscle.

Pool and Sonnenblick (62) investigated isolated cat papillary muscle at a temperature of 25°C. They followed the iodoacetate-nitrogen blockade model of Lundsgaard (47), and found that there was a decline in CP during the resting flaccid state. With a passive stretch, the breakdown of CP was increased. Energy utilization for contractile element work in isometric muscle contractions agreed closely with that predicted by heat measurements. The efficiency of high energy phosphate utilization for mechanical work was 33 percent under the experimental conditions studied. Further studies by Pool et al (9), employing isotonic and isometric contractions, demonstrated that in heart muscle, energy utilization could be described by an equation of the form:

$$\Delta \sim P = K_0 + K_1A + K_2W_i + K_3W_e, \qquad \text{IV}$$

where $\Delta \sim P$ is the change in high energy phosphate, A is the number of activations, W_i is the work of the contractile element in stretching the series elastic component, and W_e is the external work performed. K_0, the intercept of the equation, did not differ significantly from zero. The mean of K_1 was 0.1047 μmoles $\sim$ P/g per minute, and for K_2 and K_3 the means were 0.0067 and 0.0031 μmoles/g-cm. Thus, internal work (tension development) appears to consume more energy than external work (shortening against a load). The term K_1A incorporates all the time related energy consuming processes represented by activation plus basal energy. By subtracting the basal energy of the resting muscle, the energy cost of activation was found to be 0.040 μmoles/g per activation. These findings with high energy phosphate analysis are similar to the relationships of tension and flow work with oxygen consumption and heat. As in skeletal muscle investigations, no chemical energy equivalent to the shortening per se could be proven. Figure 2 shows these relationships as the load on muscles is increased from zero, when maximum shortening occurs, up to a load large enough to produce an isometric state. The maximum external and total work occur at about one-half isometric load. With further increase in the load, total and external work appear to decline, while internal work continues to increase. The changes in high energy phosphate utilization are similar to the changes seen with heat and oxygen consumption when load is increased.

Further similarities between oxygen consumption, heat, and high energy phosphate utilization by the heart muscle were demonstrated when the energy relationship to the inotropic state was studied. Chandler, Sonnenblick, and Pool (63) found that isolated cat papillary muscle used more energy in the presence of norepinephrine than control muscles performing the same amount of work. This increased energy cost of work was related to the greater inotropic state of the muscle.

Thus, all the known mechanical factors that influence oxygen consumption and heat production in heart muscle similarly effect high energy phosphate utilization. However, oxygen consumption, high energy phosphate utilization, and CEW must be known within any one system in order to determine the efficiency of converting oxygen to high energy phosphate and high energy to mechanical activity. Heat is needed to measure the inefficiency of these processes (64).

Energy in Abnormal States

The most significant clinical condition relating to altered energy metabolism is that of decreased oxygen delivery to the myocardium, as is seen in ischemic heart disease.

Severe hypoxia of the heart is associated with an abrupt fall in the content of high energy phosphate compounds (65, 66). It has been assumed that reduction in high energy phosphate levels is responsible for the loss in contractility in myocardial ischemia. However, when hypoxia or ischemic heart failure is produced, signs of left ventricular failure occur before a detectable decline in subepicardial high energy phosphate levels (67, 68). It has been suggested that energy stores in the subendocardial portion of the myocardium are more susceptible to ischemia, and that altered metabolism in this region may be responsible for the mechanical changes (69). This would be consistent with the finding of decreased oxygen tension and coronary flow distribution to the subendocardium when coronary flow is reduced (70, 71). If whole myocardial high energy stores are studied, when hypoxia is imposed while cardiac work continues at a high load, mechanical changes parallel depletion and repletion of high energy phosphates quite closely (66). This is particularly true when the release of endogenous catecholamines are taken into account. During the first minute of hypoxia, left ventricular systolic pressure increases. When hearts of animals treated with reserpine are studied, this increase is abolished and the time course of left ventricular systolic pressure and high energy phosphate declines are similar. If cardiac work is allowed to diminish abruptly when hypoxia is imposed, high energy stores fall minimally (72), suggesting that the interaction of hypoxia and work is necessary before high energy phosphate changes can be demonstrated.

When a portion of the heart is made ischemic, the force of contraction of that segment declines, and finally expansion occurs during systole (73). If a large enough portion of the heart is affected, there will be a decrease in ventricular function and failure will ensue (74). The noncontractile portion of the heart may serve as an added elastic component, against which the remainder of the myocardium must contract in order to develop enough pressure to open the semilunar valves and eject blood. In this state, each surviving contractile element must shorten farther in order to take up this added elastic element during the isovolumic contraction phase. In addition to these mechanical burdens, dilatation of the ventricle frequently occurs. These changes cause an increase in magnitude and duration of ventricular force required to open the semilunar valves and propel blood into the aorta. Because CEW is increased, a disproportionately large amount of energy is expended for a given cardiac output. The most pronounced mechanical burdens of these types are seen with large myocardial infarctions (74) or with ventricular aneurysms (75). Recognition of the nature of these mechanical disadvantages has led to more aggressive surgical approaches for removal of aneurysms or large myocardial infarctions (76, 77).

Effects of Increased Mechanical Load (Valvular Lesions or Hypertension)

The relationship between energy availability and mechanical function in congestive heart failure due to valvular disease is a complex subject. Two hemodynamic situations must be considered. In conditions such as in hypertension and aortic or pulmonic stenosis, there are greater pressure loads, resulting in a need for greater tension development by the CE. When valvular regurgitation is present, the predominant hemodynamic abnormality is one of increased flow or CE shortening. Combinations of these two states are frequent. Because methods for determining cardiac metabolic parameters in the intact human are inaccurate, important quantitative differences in myocardial energetics in humans have been difficult to document. Oxygen consumption per gram myocardium has been usually found to be normal or slightly increased when patients with valvular disease, with or without congestive heart failure, have been studied (78–81). However, since cardiac mass is usually increased, the oxygen cost for a given amount of cardiac output may be quite high. When dilatation occurs, the rate of development of tension by the contractile element and the rate of fiber shortening are slow, and peak tension and velocity occur later in systole (82). Furthermore, because of the increased ventricular size, the amount of contractile element work to develop the same pressure is increased.

In experimental animals, the energetics of contraction in abnormal states have been determined with more precise techniques. The contractile function of myocardium is subnormal in hearts in which hypertrophy and failure have been induced by pressure overload (83). The level of CP may also be found to be reduced under these circumstances, and this may be secondary to greater total metabolic demands (84). However, ATP levels and the high energy phosphate cost of contractile work is not increased in muscle from these hearts (85). Thus, myocardial failure of the pressure overload type appears not to be due to inefficient coupling of chemical to mechanical energy. Other evidence suggests that a defect in myocardial energy production is also not responsible for failure in the overloaded heart (86, 87). Even though these basic relationships appear to be normal, in the intact heart with an increased hemodynamic burden, the force required during the isovolumic and ejection phases will be greater. This, in addition to the increased muscle mass, the prolongation of tension, and ventricular dilatation, all may contribute to increased energy requirements (30).

As predicted from data of Evans and Matsuoka in 1914 (26), when volume overload predominates, the external work of the heart is also increased. When acute or chronic aortic regurgitation is produced experimentally, slight increases in myocardial oxygen consumption are observed (88, 89). Aortic and mitral regurgitation in the dog do not alter the inotropic state of the heart, but are associated with increased ventricular volume, greater magnitude and velocity of contractile element and fiber shortening, and peak wall tension (90). Marked degrees of regurgitation may occur with only slight increases in the oxygen consumption. Most of the increment in oxygen consumption is accounted for by the increased tension. Thus, when tension is held constant, no change in oxygen consumption is observed with acute regurgitation. In clinical states in which chronic regurgitation is present, considerable degrees of cardiac dilatation frequently are associated with cardiac hypertrophy. As noted above, this will require more tension development, and therefore more energy consumption. This abnormality may be difficult to demonstrate at rest, but will become accentuated during mild exercise if the heart dilates even further.

The Heart in Thyroid Disorders

Increased myocardial oxygen consumption per beat has been demonstrated in humans with thyrotoxicosis (91). This suggests that thyroid hormone may have a direct effect upon myocardial energetics. Contractility has been found to be increased in papillary muscles from hyperthyroid cats (92). Studies in humans indicate also that hyperthyroidism and hypothyroidism are associated with increased and decreased cardiac inotropy, respectively (93). Thus, it would be expected that energy utilization would vary in proportion to the thyroid activity.

Studies in hyperthyroid dogs have failed to demonstrate a defect in oxidative phosphorylation in the myocardium, and stores of high energy phosphate compounds are reported to be the same as in control animals (94). In isolated papillary muscles from hyperthyroid cats, both oxygen consumption and high energy phosphate utilization per unit of contractile element work have been reported to be greater than normal (95, 96). Thus, in hyperthyroidism there may be two mechanisms for abnormal energetics. One is increased inotropy, causing a greater energy demand, and the other is a decreased efficiency of conversion of chemical to mechanical work. These factors need to be clarified.

Effects of Digitalis

The effects of the digitalis glycosides upon myocardial energetic relationships probably are secondary to their influence upon myocardial work and contractility. In isolated muscle preparations, digitalis increases both the contractile element work and the velocity of contraction, and these effects of the drug have been found to be associated with increased oxygen consumption (42). Similar mechanical effects of digitalis have been observed in intact non-failing human hearts (97). In carefully controlled studies in dogs, digitalis has been found to decrease the systolic tension developed by the non-failing heart, but to simultaneously increase the velocity of contraction (98). This results in a mean increase in the oxygen consumption and a decline in external efficiency. If end-diastolic pressure and volume are made abnormally high, to simulate congestive failure, digitalis decreases these so that tension falls to a greater extent and oxygen consumption is not altered. Thus, whereas digitalis in the non-failing heart may be associated with a decreased metabolic to mechanical efficiency as expressed by cardiac output, in the failing heart digitalis may promote a more favorable geometry for efficient conversion of chemical to mechanical energy.

Summary and Conclusions

In this review we have outlined the important mechanical factors related to heat production, oxygen consumption, and high-energy phosphate metabolism in the myocardium. We have attempted to express the mechanical determinants of energy utilization in terms of the work of the contractile element and the inotropic state (contractility) of the myocardium and intact heart. We have also noted how these principles apply to cardiac function during ischemia, in myocardial infarction, when heart failure due to hemodynamic overload is present, with thyroid abnormalities, and with digitalis therapy.

Although skeletal muscle energetics have been studied for almost a century, similar investigations in cardiac muscle and the intact whole heart have only been recently begun. In order to calculate absolute chemical and mechanical efficiencies, simultaneous measurements of mechanics, heat production, high-energy phosphate utilization, and oxygen consumption must be performed. The technical problems in such studies are formidable. How these relationships vary with different loads and in different inotropic states, and the effect of normal and abnormal energy levels in the myocardium need further investigation.

The relationship between energy supplies and decreased function of hypoxic myocardium remains unexplained, and it awaits further clarification. Although there is evidence of increased inotropic state in hyperthyroidism, the possibility of decreased conversion of energy to mechanical work must be investigated further.

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Stop and Look ... Before You Listen: Non-Cardiac Clues to the Diagnosis of Cardiovascular Disease

NANETTE KASS WENGER, M.D.†

Introduction

"There is a great deal in first impressions," commented William Congreve in The Way of the World (1). In cardiology, as in the play, it is often a first impression from meticulous inspection of the patient that provides the etiologic clue to an otherwise obscure cardiovascular lesion.

Congenital Heart Disease

In mongolism, or Down's syndrome (2) (Fig. 1), the complex of slant eyes, congenital cataracts, protruding tongue, epicanthal folds, Brushfield spots of the iris, and a simian crease in small radially deviated hands warn of the possibility of endocardial cushion defect, patent ductus arteriosus, and mitral and tricuspid valve defects.

The patient with Turner's syndrome (gonadal dysgenesis) (3) (Fig. 2) with webbing of the neck, short stature, hyperteleorism, increased carrying angle of the elbows, broad shield chest with widely separated nipples, and the absence of breast development is likely to have congenital heart disease. Coarctation of the aorta is most common in the female, atrial or ventricular septal defect in the male, and both may have pulmonic stenosis.

The child with the Gregg-Swan syndrome (4) (Fig. 3), evidence of *in utero* rubella infection, classically has congenital cataracts, eighth nerve deafness, microphthalmos, microcephaly, and mental retardation as the systemic associates of patent ductus arteriosus and pulmonic stenosis; coarctation of the pulmonary arteries is also likely.

The characteristic facies of hyperteleorism, pouting lips, prominent chin and nose, hypoplasia of the mandible, a dull facial expression, and strabismus are overt signs of supravalvular aortic stenosis (5) (Fig. 4); these patients are also likely to have coarctations of the pulmonary arteries.

In Noonan's syndrome (6), the identification of hyperteleorism, low set ears, an anti-mongoloid slant to the eyes, small stature, mild mental retardation, and simian creases of the palms suggests that the cardiac lesion is valvular pulmonic stenosis.

The murmurs of mitral or tricuspid valve disease should be sought for in the patient with Hurler's syndrome (gargoylism) (7) (Fig. 5), who presents the clinical picture of dwarfism, deafness, multiple skeletal abnormalities, corneal clouding, macroglossia, hirsutism, hepatosplenomegaly, and mental retardation. In these patients, endocardial fibroelastosis extends onto the atrio-

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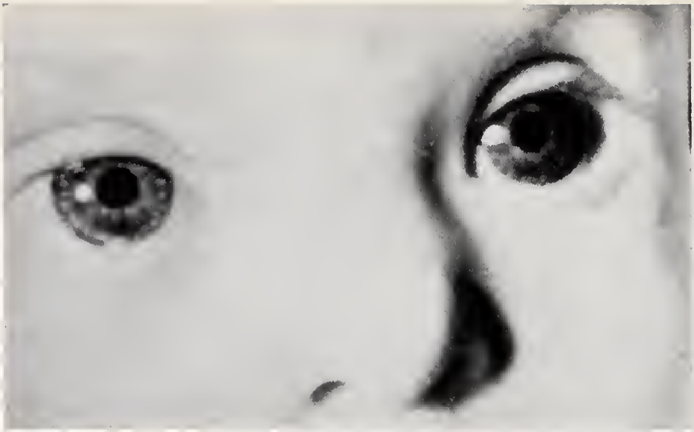

Fig. 1. Mongolism (Down's syndrome). Brushfield's spots in the iris and epicanthal folds are apparent. Atrial septal defect and endocardial cushion defects may be present in such patients.

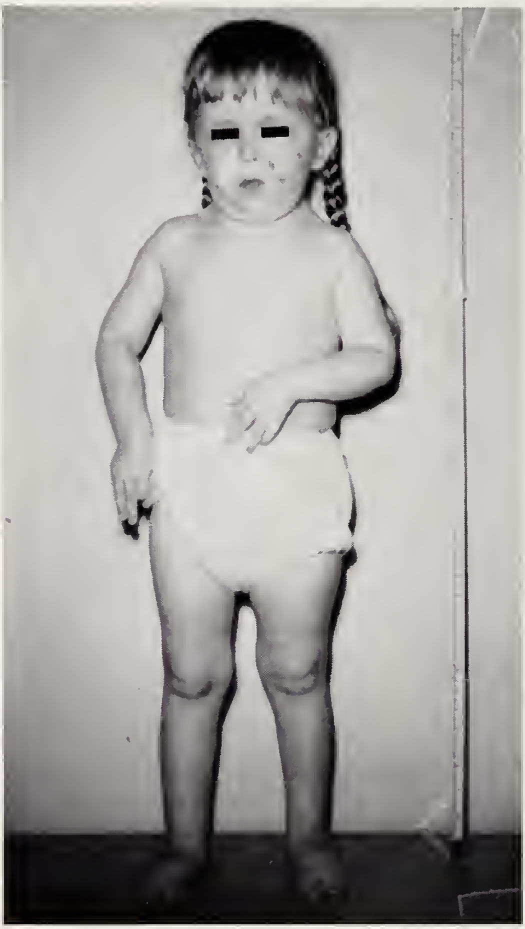

Fig. 2. Turner's syndrome. Note the webbed neck and shield chest (the eyes had an antimongoloid appearance). These patients have a high incidence of coarctation of the aorta and pulmonary stenosis.

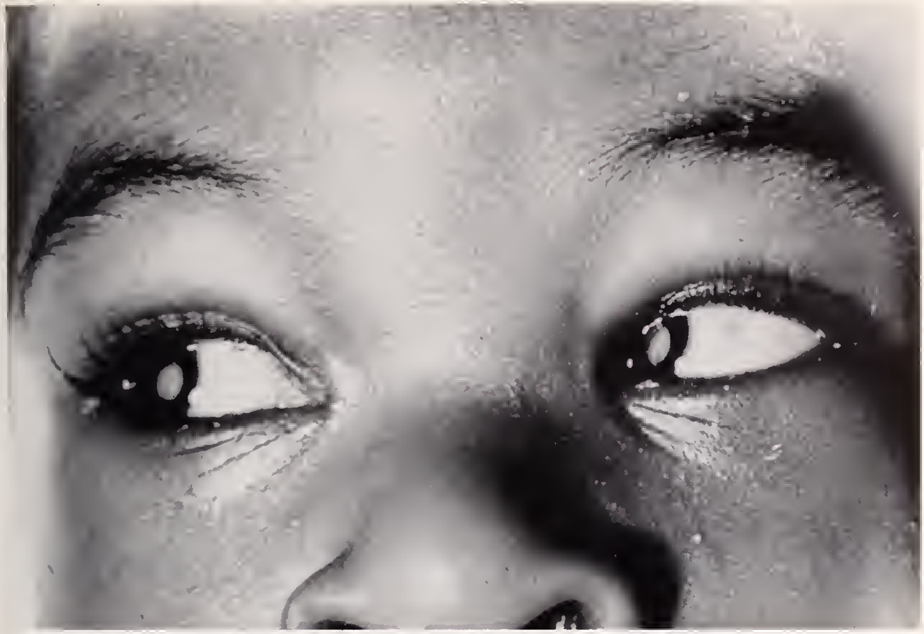

FIG. 3. Gregg-Swan syndrome. Note cataracts. Patent ductus arteriosus with or without pulmonary stenosis is common in these patients. Coarctation of the pulmonary artery may occur.

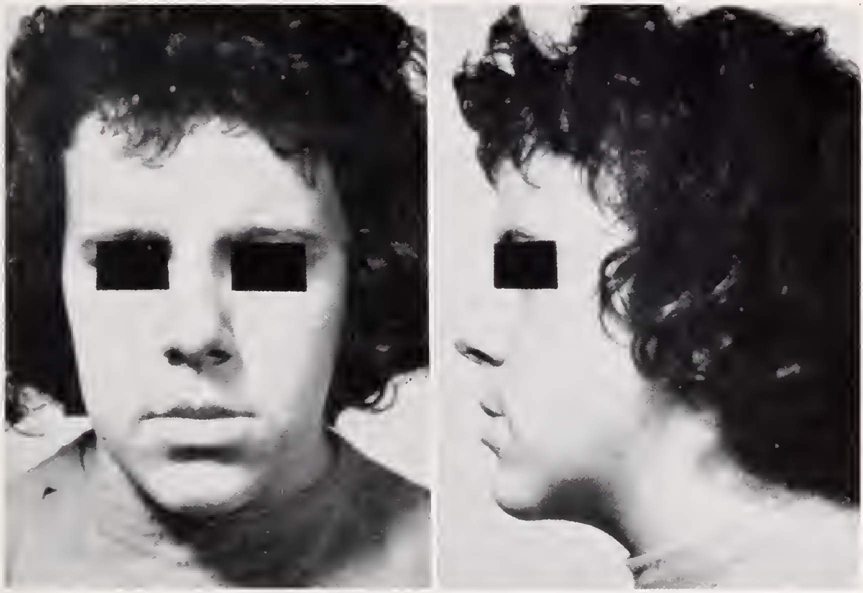

FIG. 4. Photographs of the facies of a patient with supravalvular aortic stenosis show a long upper lip, full cheeks with prominent chin, up-turned nose, and full, pouting lower lip. Strabismus, seen in some cases, was not present in this patient.

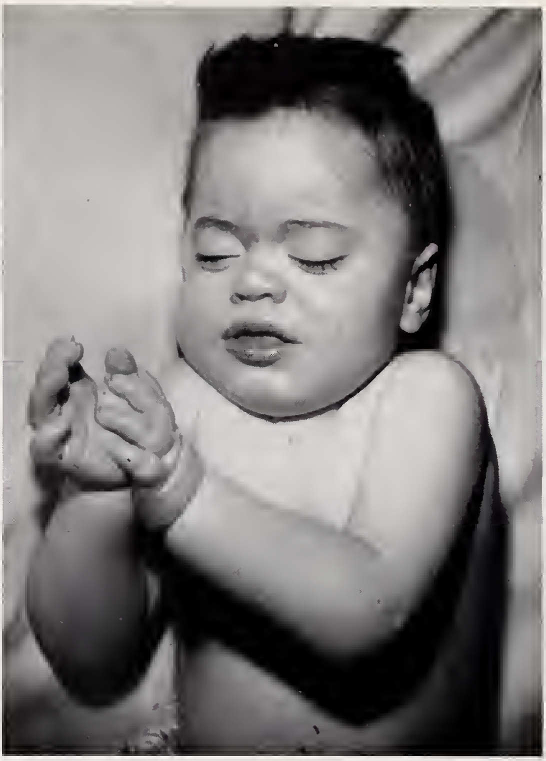

FIG. 5. Hurler's syndrome. Note the coarse features, bushy eyebrows, and short, stubby hands. Abnormal amounts of mucopolysaccharides may be deposited in the heart valves, coronary arteries, and aorta of such patients.

ventricular valves with resulting hemodynamic impairment; patients with gargoylism also have deposition of a macromolecular glycoprotein causing coronary artery narrowing and a cardiomyopathy.

The Ellis-van Creveld syndrome (8, 9), manifest by dwarfism due to short extremities with more distal than proximal shortening, polydactyly, and chondroectodermal dysplasia with hypoplastic nails and peg-shaped teeth, is a clue to the cardiac diagnosis of a large atrial septal defect or a single atrium; ventricular septal defect also occurs.

In the Laurence-Moon-Biedl-Bardet syndrome (10), the possibilities of patent ductus arteriosus, transposition of the great vessels, pulmonic stenosis, dextrocardia, or a hypoplastic aorta are suggested by the combination of polydactyly, obesity, mental retardation, hypogonadism, and retinitis pigmentosa.

Ventricular septal defect and dextrocardia are the cardiac concomitants of the trisomy 13–15 (D_1) syndrome (11), which presents as polydactyly, a simian palmar crease, cleft palate, microphthalmia, and hemangiomas.

The hallmarks of the trisomy 16–18 (E_1) syndrome (12) (Fig. 6) include a tightly clenched fist with the index finger overlapping the third finger and

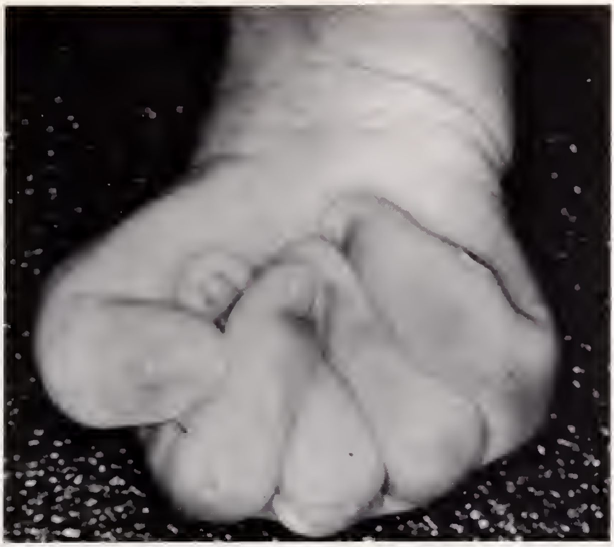

FIG. 6. E_1 trisomy (group 16 to 18) syndrome. Typical posturing of the hands occurs in these patients. A variety of cardiac abnormalities has been reported with this syndrome.

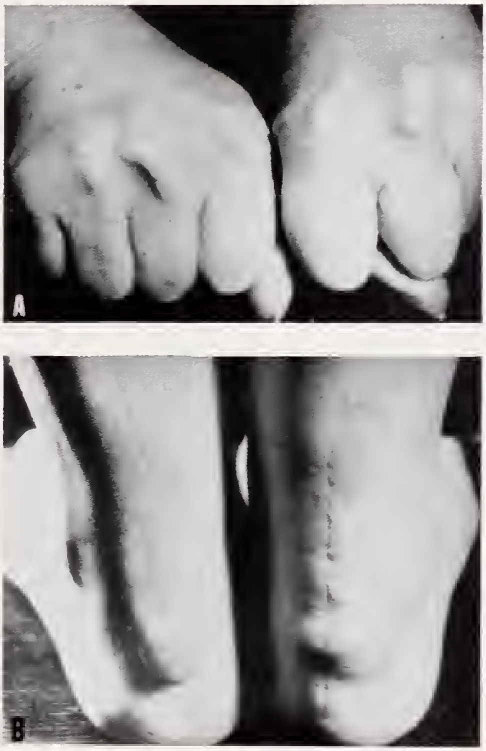

FIG. 7. Xanthomatosis. A. Xanthomatous nodules located on the tendons of the hands; B. large xanthomatous masses located in the Achilles tendons. This patient was a middle-aged female with aortic stenosis and coronary artherosclerosis.

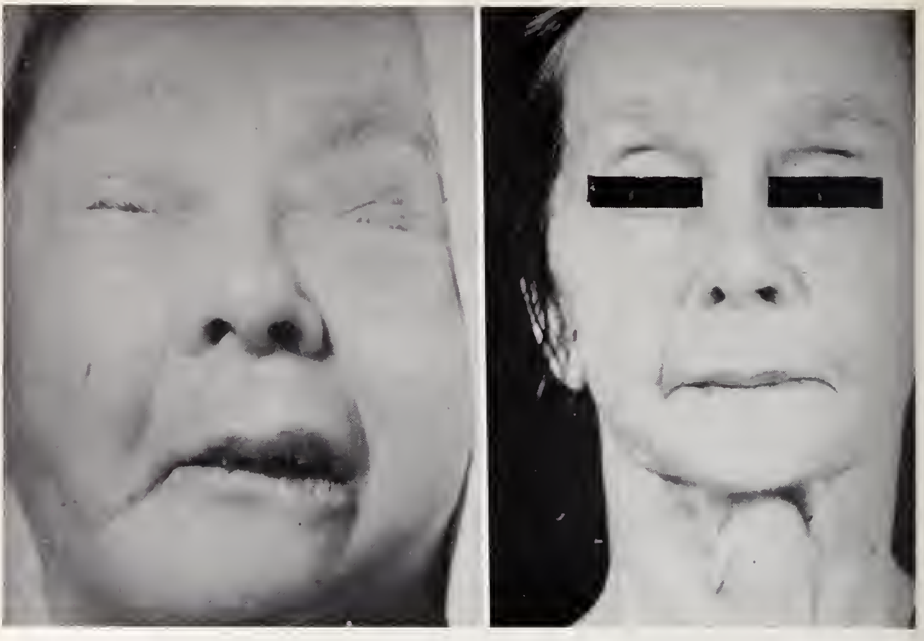

Fig. 8. Myxedema. Patient in near-coma shows typical facies (left). Some months after thyroid therapy (right). Pericardial effusion is common in this disease, and is the major cause of the large cardiac silhouette seen on the x-ray.

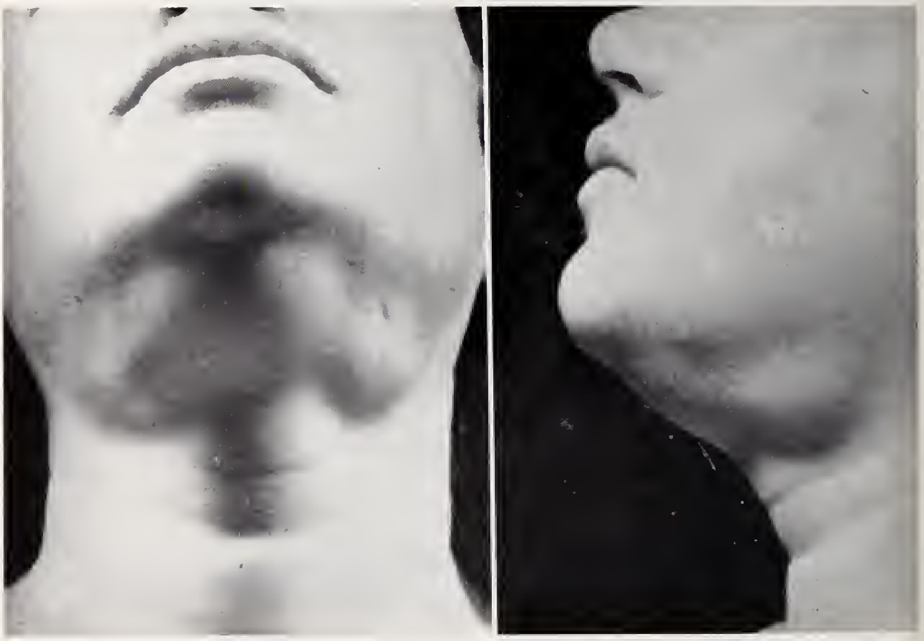

Fig. 9. Amyloidosis. Macroglossia may be due to amyloid infiltration of the tongue. This young woman had amyloid deposits in the base of the tongue. This depressed the submaxillary glands that are seen in the photograph.

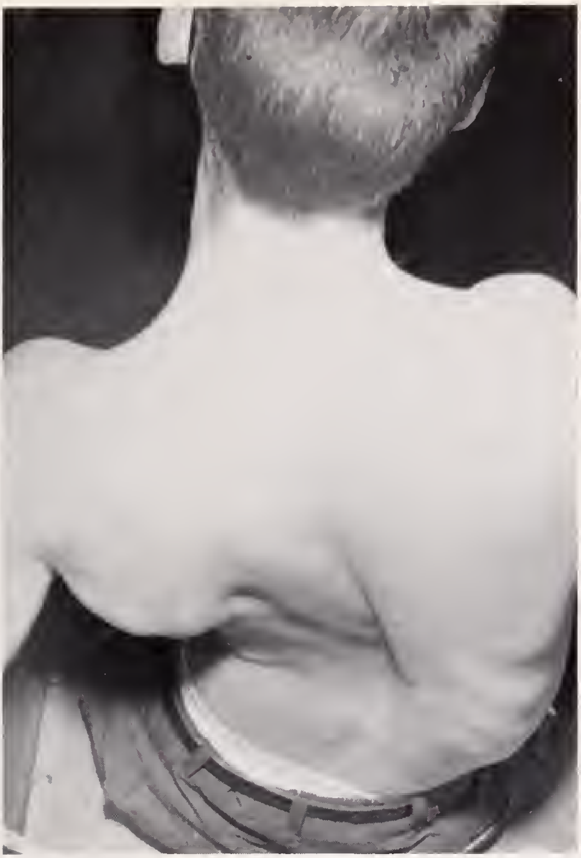

FIG. 10. Kyphoscoliosis secondary to Friedreich's ataxia. This young boy developed marked cardiac enlargement of the type seen in Friedreich's ataxia before kyphoscoliosis developed.

the fifth finger overlapping the fourth, micrognathia, a short webbed neck, flexion deformity of the arms, and ear anomalies; a ventricular septal defect, patent ductus arteriosus, or abnormal cardiac valves are common.

Atrial septal defect is the usual cardiac defect in the Holt-Oram syndrome (13, 14), in which the major clinical manifestation is a hypoplastic fingerized thumb; other upper extremity bony abnormalities may be observed.

Absence or hypoplasia of the radius and a fingerized thumb, ventriculoradial dysplasia (15), should raise the suspicion of a ventricular septal defect.

Broad thumbs and great toes, low set ears, an anti-mongoloid slant to the eyes, and mental retardation characterize the Rubinstein-Taybi syndrome (16), which has patent ductus arteriosus, double aortic arch, and pulmonic stenosis as the cardiac concomitants.

The constellation of multiple long bone deformities secondary to fractures, blue sclerae, deafness, and relaxed tissues suggests the cardiac diagnosis of aortic regurgitation; the disease is osteogenesis imperfecta (17).

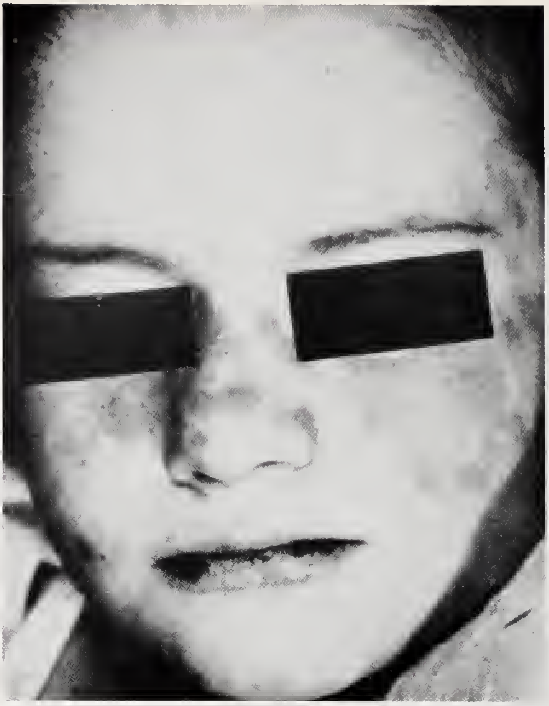

Fig. 11. Young girl with lupus erythematosus. Typical butterfly rash of the face. The rash may not be so obvious, or may not be present at all. Patients with lupus erythematosus may have myocarditis and pericarditis.

A short, immobile webbed neck due to fusion of the cervical vertebrae, and a low hairline occur together with ventricular septal defect and, less commonly, with transposition of the great vessels or atrial septal defect in the Klippel-Feil syndrome (18).

Acquired Valvular Disease

Episodic skin vasomotor phenomena, bronchoconstriction, telangiectasia, anxiety, abdominal cramps, and diarrhea are linked with the murmurs of tricuspid or pulmonic valve disease in the patient with the carcinoid syndrome (19, 20).

The recognition of a poker spine characteristic of ankylosing spondylitis (21) should prompt a search for the murmur of aortic regurgitation. Similarly, the recognition of psoriasis may provide an explanation for aortic regurgitation (22).

Aortic regurgitation is also a salient feature of relapsing polychondritis (23), identifiable by collapse of the bridge of the nose, cauliflower ears, interstitial keratitis, and erythema, with swelling and destruction of the collagenous tissues.

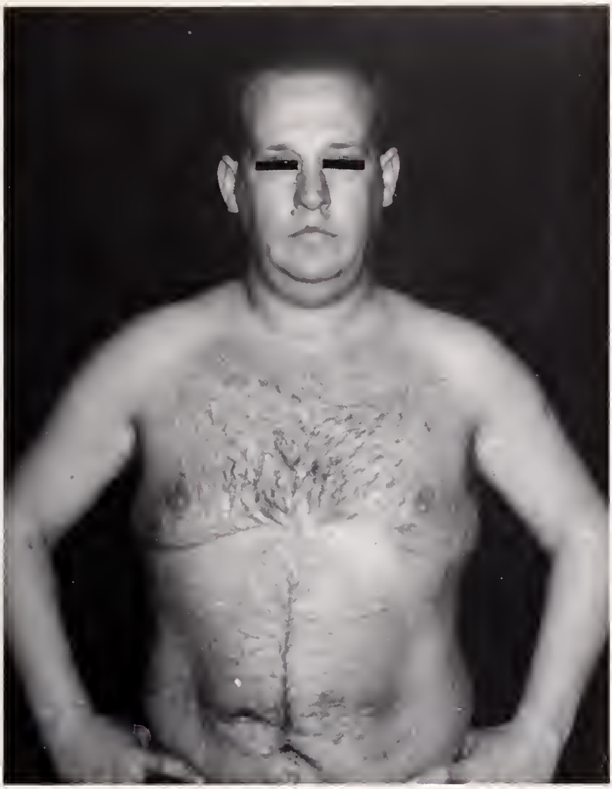

Fig. 12. Cushing's syndrome. Note the absence of typical moon facies. The striae on the lateral aspects of the upper portion of the abdomen suggest the diagnosis.

Disease of the Coronary Arteries

The shoulder-hand syndrome (24), with a swollen, discolored, shiny, stiff hand, may be evidence that a myocardial infarction has occurred subsequent to atherosclerotic disease of the coronary arteries; a Dupuytren's contracture (25), with palmar thickening and nodules, similarly suggests a prior myocardial infarction.

The patient with xanthomatous deposits in the Achilles tendon, elbows, or fingers, and xanthelasma as evidence of familial hypercholesterolemia (26) (Fig. 7) is prone to premature coronary atherosclerosis; there may be associated aortic valvular stenosis.

A dull, puffy facial expression, periorbital edema, macroglossia, a dry coarse skin, hoarse slow speech, and slow relaxation of the deep tendon reflexes should suggest the diagnosis of myxedema (27, 28) (Fig. 8). These patients have hypercholesterolemia and have a propensity for myocardial infarction, but also may present with enlargement of the cardiac silhouette due to pericardial effusion.

A propensity for coronary atherosclerosis is seen in the patient with acro-

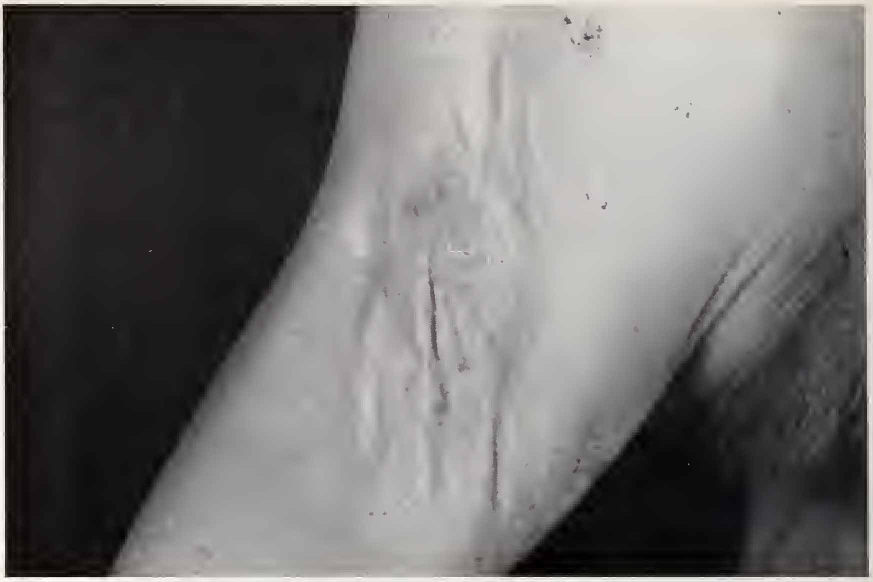

FIG. 13. Pseudoxanthoma elasticum. The skin is crepe-like, lax, redundant, and inelastic. Arterial calcification, hypertension, and gastrointestinal hemorrhage occur in these patients.

megaly (29, 30), who may show gigantism, prognathism, and spade-like hands with short, broad fingers. Additional cardiac abnormalities include cardiac enlargement, congestive heart failure, conduction defects, and myocardial hypertrophy and fibrosis.

Premature coronary atherosclerosis is also associated with the Werner syndrome (31, 32), clinically apparent as skin atrophy with loss of subcutaneous tissue, cataracts, osteoporosis, and multiglandular dysfunction, particularly hypogonadism.

The patient with homocystinuria (33) classically has long extremities, arachnodactyly, lax joints, dislocation of the lens, kyphoscoliosis, and pectus carinatum. The usual cardiovascular lesions are thrombosis of the medium-size coronary arteries, which may produce myocardial infarction and thrombosis of peripheral arteries, which may be manifest as intermittent claudication. These patients have mental retardation and cutaneous flushing.

Myocardial Disease

Waxy deposits in the skin folds of the neck or perineum may be a sign of amyloidosis (34, 35) (Fig. 9); ecchymoses are also found and macroglossia may be prominent; the patient may suffer with atrial arrhythmias and a restrictive cardiomyopathy.

Large calves, a waddling gait, and difficulty in assuming the upright posture, with a "climbing up the legs" phenomenon are the external changes that may accompany the cardiomyopathy and often distinctive electrocardiographic abnormalities associated with Duchenne's muscular dystrophy (36, 37).

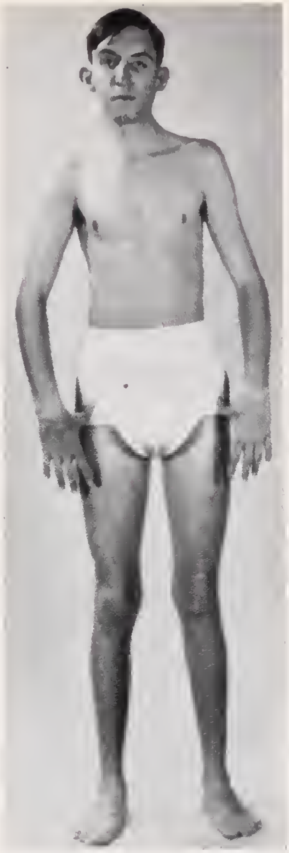

FIG. 14. Absence of subcutaneous tissue, frail muscular development, and scoliosis in this young man with aortic insufficiency and cleft palate are characteristic of Marfan's syndrome.

The combination of kyphoscoliosis and other skeletal deformities, ataxia, and speech disturbance should alert one to the diagnosis of Friedreich's ataxia (38, 39) (Fig. 10); this furnishes a ready explanation for the cardiac enlargement, heart failure, arrhythmias, and electrocardiographic abnormalities.

The patient with the expressionless face, frontal balding, cataracts, gonadal atrophy, slow muscle relaxation after contraction, and muscle atrophy presents a clue to the etiology of the cardiomyopathy association with myotonia atrophica (40); arrhythmias and an abnormal electrocardiogram may also occur.

The infant with the characteristic findings of macroglossia, areflexia, neurologic defects, and profound muscle weakness has Pompe's (glycogen-storage) disease (41, 42) as the etiology of the cardiomyopathy; the electrocardiographic abnormalities of massively increased QRS voltage and a short PR interval are typical.

Subcutaneous nodules, transient arthritis, erythema nodosum, and alopecia may reflect sarcoidosis (43, 44) and furnish an explanation for the tachy-

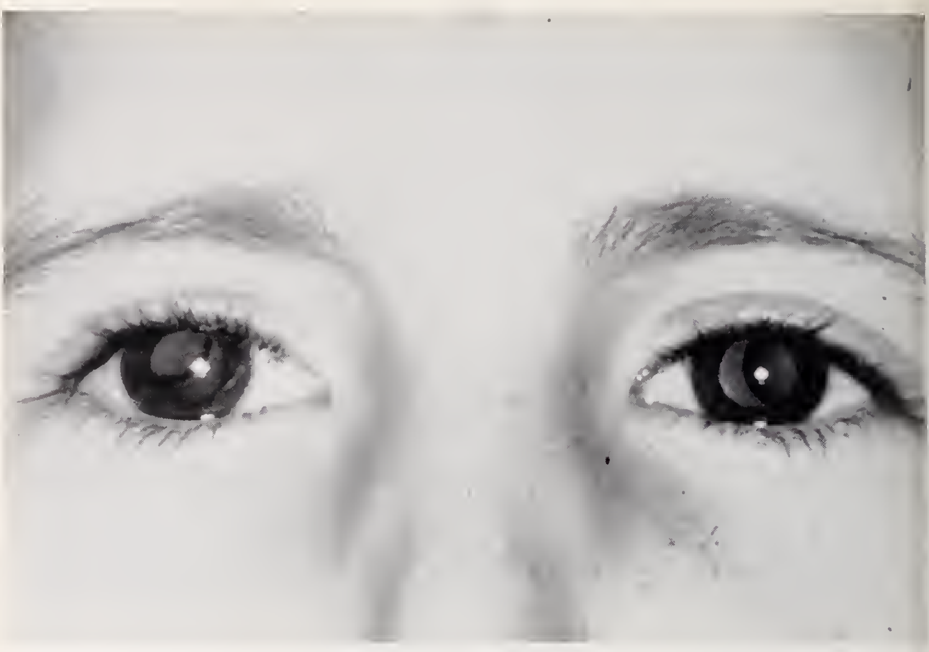

FIG. 15. Dislocation of lens of the eye in another patient with Marfan's syndrome.

cardia, cardiac arrhythmias, or conduction abnormalities, cardiac enlargement, congestive heart failure, cor pulmonale, or pericarditis.

The patient with scleroderma (45), evidenced by a mask-like facies, skin erythema, induration and depigmentation, Raynaud's phenomenon, with pallor and cyanosis of the fingers, may be subject to diffuse myocardial fibrosis. There may be accompanying pericarditis, valve thickening, and cor pulmonale secondary to pulmonary scleroderma.

Similarly, dermatomyositis (46) may involve the myocardium and the pericardium; the typical patient's appearance is of muscle weakness with atrophy, contraction of the hand, and a scaly skin rash.

A butterfly rash of the face, vitiligo, hyperpigmentation, palmar macules, painful swollen interphalangeal joints, and Raynaud's phenomenon are the overt signs of lupus erythematosus (47) (Fig. 11); diffuse involvement of the myocardium occurs, often with conduction defects. This may be accompanied by verrucous endocarditis and acute or chronic pericarditis.

The superficial manifestations of hemochromatosis (48, 49) include a bronze or slate-gray skin pigmentation, hepatosplenomegaly, testicular atrophy, and a history of diabetes mellitus; these have a striking connection with iron pigment infiltration of the myocardium with resultant arrhythmias, conduction disturbances, cardiac failure, and often pericarditis.

Rheumatoid arthritis (50, 51), identified by ulnar deviation of the hand, boxing of the wrists, and inflammation of the interphalangeal joints may be associated with granulomatous myocarditis; granuloma may also involve the aortic and mitral valves, and there may be acute or chronic pericarditis.

The patient with the Pickwickian syndrome (obesity-hypoventilation) (52) presents with cyanosis, tachycardia, extreme obesity, and somnolence; the salient cardiac features include pulmonary hypertension and right ventricular failure.

Hypertension

In the patient with pheochromocytoma (53), the combination of excessive sweating, pallor, tremulousness, café au lait spots, and neurofibromatosis is the initial clue that the fixed or paroxysmal hypertension may be curable.

Curable hypertension, with concomitant cure of the frequently associated cardiac failure, should be suggested by the classical presentation of Cushing's syndrome (54, 55) (Fig. 12): truncal obesity, buffalo hump, purplish thoracic and abdominal striae, easy bruising, hirsutism, moon facies, and acne.

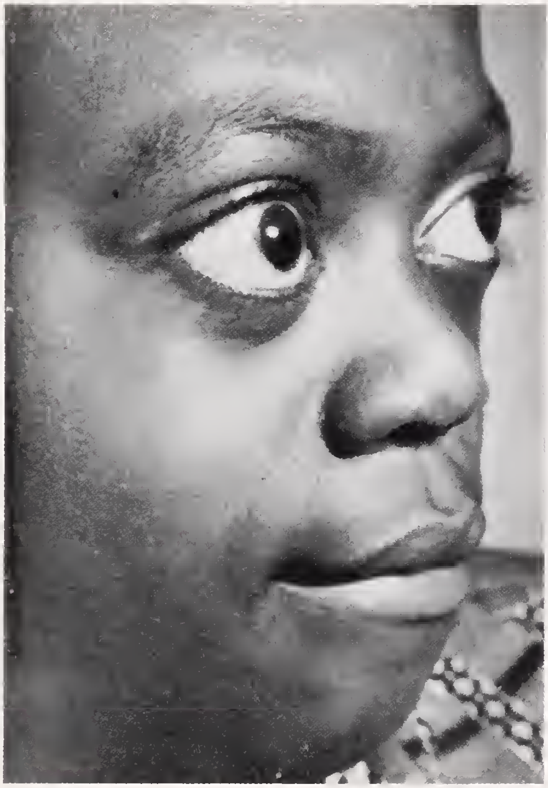

Fig. 16. Thyrotoxicosis. Thyrotoxicosis may occur at any age. This photograph shows typical exophthalmos in a young boy. Thyrotoxicosis in the older age group may not be so obvious. Atrial fibrillation is frequently due to thyrotoxicosis. Thyrotoxicosis may precipitate or aggravate heart failure and angina pectoris.

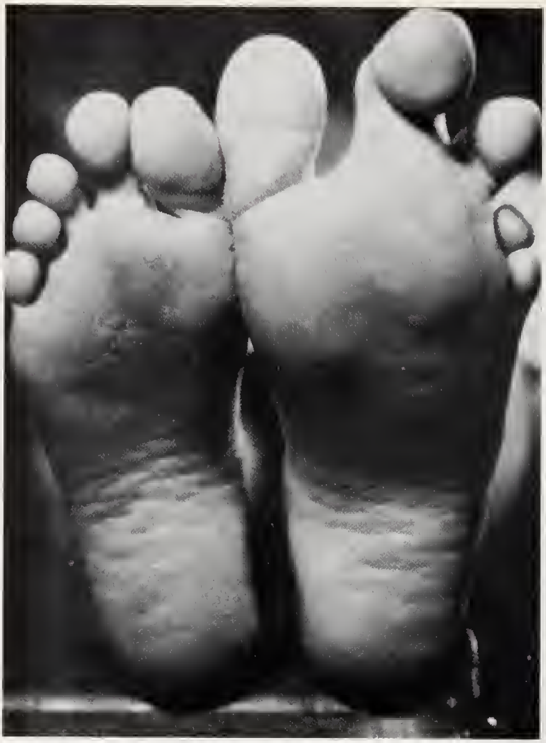

FIG. 17. Congenital peripheral arteriovenous fistulas. Asymmetric enlargement of an extremity or digit associated with unusual varicose veins may occur in patients with congenital peripheral arteriovenous fistulas. The grotesque enlargement of the toes and foot on the right is obvious.

Profound muscle weakness and polyuria should direct attention to hyperaldosteronism (56) as an etiology for hypertension.

Crepe-like skin lesions are an indication of pseudoxanthoma elasticum (57) (Fig. 13); hypertension, gastrointestinal hemorrhage, and angioid streaks of the retina occur commonly.

Disease of the Aorta

Dissecting aneurysm of the aorta has a striking connection with the Marfan's syndrome (58, 59) (Figs. 14 and 15). Classically, the patients are tall and asthenic, with arachnodactyly, pectus excavatum or carinatum, kyphoscoliosis, lax joints, a high narrow arched palate, and subluxation of the lens. Other cardiovascular abnormalities may include saccular aneurysms of the aorta, pulmonary artery, or the sinus of Valsalva; aortic regurgitation; mitral and occasionally tricuspid regurgitation; redundant chordae tendineae; and disease of the small coronary arteries.

Dissecting aneurysm of the aorta also occurs in the Ehlers-Danlos syndrome (60), which is clinically apparent as hyperelastic skin, cigarette-paper scars due to friable connective tissue, and hyperextensible joints. Additionally, these patients may have spontaneous arterial rupture and/or the apical systolic murmur of mitral regurgitation due to redundant chordae tendineae.

Cardiac Tumors

Rhabdomyoma of the heart should be suspected in the patient with tuberous sclerosis (61, 62); salient features on inspection include epilepsy, café au lait spots, mental retardation, and adenoma sebaceum.

Clubbing of the fingers in the absence of cyanosis may be due to left atrial myxoma (63); dyspnea or syncope in the upright position is a tell-tale sign for this diagnosis.

Electrocardiographic Abnormalities

The patient with pectus excavatum (64) may be referred to the cardiologist because of nonspecific electrocardiographic abnormalities, and the patient with the straight back syndrome (65, 66), because of Q waves or RSR' complexes in the right precordial leads. Both of these conditions are also characterized by ejection systolic murmurs over the right ventricular outflow tract and pseudocardiac enlargement on the PA film of the chest. Once the skeletal abnormalities are identified, these patients may be "delabeled," in that their murmurs and electrocardiographic and radiologic abnormalities have mimicked cardiac disease.

A deaf-mute (67) patient who reports dizziness and syncope may be prone to sudden death; the electrocardiographic associations include a prolonged QT interval and T wave abnormalities.

The leopard syndrome (68), a mnemonic name, identifies the components of lentigines, electrocardiographic defects, ocular hyperteleorism, pulmonic stenosis, abnormalities of growth, retardation of growth, and deafness; electrocardiographic abnormalities include abnormal P waves, 1° AV block, QRS widening and bundle branch block, and ST-T changes.

High-Output Cardiac Failure

High-output cardiac failure has a striking connection with thyrotoxicosis (69, 70) (Fig. 16); the overt clinical signs include quick movements, a tremor, widened palpebral fissures, a stare, and warm moist skin; other frequent cardiac findings include sinus tachycardia or atrial fibrillation and angina pectoris.

Congenital arteriovenous fistulas (71) (Fig. 17), manifest externally by hemangiomas, port wine-stains, and excessive asymmetric growth of one extremity, may also be responsible for high-output cardiac failure.

Similarly, in hereditary hemorrhagic telangiectasia (72) (Fig. 18), pulmonary arteriovenous fistulas may produce high-output cardiac failure; some interesting physical findings on inspection include telangiectasia of the lips, tongue, fingers, and nasal mucosa.

Spider angiomata, liver palms, and asterixis may signify that the high-

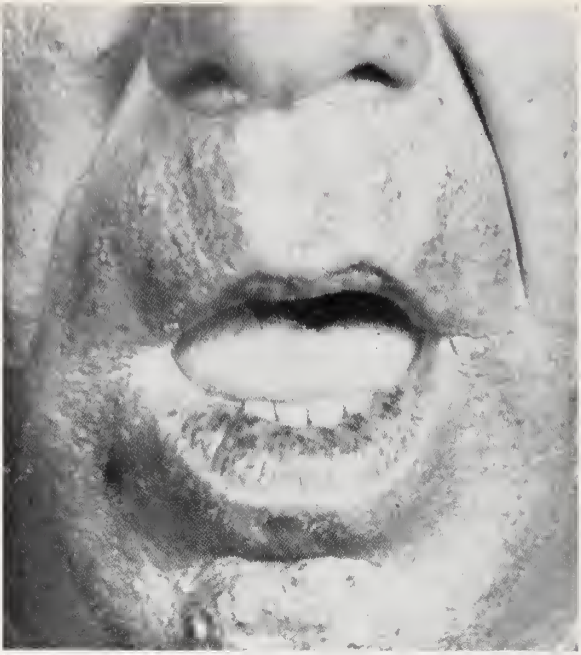

FIG. 18. Clusters of characteristic telangiectasia on the lips provided a clue to the high-output cardiac failure.

output cardiac failure is due to hepatic cirrhosis (73). High-output cardiac failure may also result from psoriasis (74), characteristically in association with severe diffuse skin lesions.

Any scar should prompt the search for a continuous murmur of a traumatic arteriovenous fistula (75) as the explanation of high-output cardiac failure.

Summary

The abundance of non-cardiac clues to the diagnosis of cardiovascular disease suggests that the physician would do well to "stop and look . . . before you listen."

Acknowledgment

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COURSES FOR GENERAL PRACTITIONERS

Office and Institutional Management of Patients Suffering from Stroke February 18, 1970 City Hospital Center at Elmhurst	Lawrence J. Wisham, M.D., Lawrence I. Kaplan, M.D., Beatrice Kaplan, M.D. and Associates Wednesday, 9:00 AM to 3:00 PM Fee: None
Hypnosis for the Physician and Dentist March 5 to May 7, 1970 The Mount Sinai Hospital	Elliot N. Wineburg, M.D. Thursdays, 4:30 PM to 6:00 PM Fee: $75
Clinical Neurology March 16 to March 20, 1970 The Mount Sinai Hospital	Morris B. Bender, M.D. and Staff Monday–Friday, 9:00 AM to 5:00 PM Fee: $75
Differential Diagnosis in Gastrointestinal Radiology March 17 to June 2, 1970 The Mount Sinai Hospital	Richard H. Marshak, M.D. and Mansho T. Khilnani, M.D. Tuesdays, 5:00 PM to 6:00 PM Fee: $50
Differential Diagnosis in Radiology of the Chest March 23 to June 8, 1970 The Mount Sinai Hospital	Coleman B. Rabin, M.D. and Bernard S. Wolf, M.D. Mondays, 5:00 PM to 6:00 PM Fee: $50
Rehabilitation of an Arthritic April 15, 1970 The Mount Sinai Hospital	Lawrence Wisham, M.D., Frances Dworecka, M.D., Beatrice Kaplan, M.D. and Associates Wednesday, 9:00 AM to 1:00 PM Fee: None
Annual Teaching Conference on Arthritis May 20, 1970 The Mount Sinai Hospital	Selvan Davison, M.D., Frances Dworecka, M.D., Emanuel Golden, M.D. and Associates Wednesday, 9:00 AM to 1:00 PM Fee: None
Care of the Patient with Chronic Stroke May 20, 1970 City Hospital Center at Elmhurst	Lawrence J. Wisham, M.D., Lawrence I. Kaplan, M.D., Mohammed Jaffari, M.D. and Associates Wednesday, 9:00 AM to 3:00 PM Fee: None

Urinary Excretion of Uric Acid after Instantaneous Intra-Renal-Arterial Injection in Mongrel and Dalmatian Dogs*

LAWRENCE BERGER, M.D., AND TS'AI-FAN YÜ, M.D.

Previous studies have revealed that the renal excretion of uric acid in various vertebrate species entails the processes of glomerular filtration and simultaneous renal tubular reabsorption and secretion (1, 2). In man and mongrel dogs, the net renal process of urate handling leads to a clearance ratio of urate to glomerular filtration rate (GFR) of less than one, the value of the ratio progressively rising with urate loading (3, 4). The large tubular reabsorptive capacity relative to the filtered load of uric acid, and to the amount eliminated in the urine, suggests that uric acid excreted in the urine derives from tubular secretion in man and mongrel dogs. In the Dalmatian dog, frank renal tubular secretion of urate can be readily demonstrated (1, 5). Pyrazinamide is capable of markedly inhibiting renal tubular secretion of uric acid in the Dalmatian dog (6) and man (7, 8, 9), but shows a lesser effect on renal tubular secretion in the mongrel dog (6, 10). In man, and even more so in the mongrel dog, the magnitude of renal tubular reabsorption of urate is much greater than that of secretion (1, 3, 4). In an attempt better to characterize the renal tubular processes involved in renal urate elimination in the dog, the technique of intra-renal-arterial injection, as described by Chinard (11), was employed in the present study. The evidence obtained suggests the presence of simultaneous processes of renal tubular reabsorption and secretion for uric acid in mongrel dogs, and again clearly shows evidence for secretion in the Dalmatian dog.

Methods

Twenty-nine experiments were performed in female mongrel dogs, and two in female Dalmatian dogs. Both ureters were exposed, and cannulated with polyethylene tubing through a suprapubic incision. The renal artery, in most cases the left, was exposed through a flank incision and punctured with a 23 gauge bent needle, to which was attached a length of polyethylene tubing. Isotonic saline solution containing 10 mg heparin per 100 ml was infused through this needle at a rate of 0.1 ml per minute, to maintain patency. In all experiments the intra-renal-arterial injection contained a total volume of 2 ml, in which in all cases 10 mg of creatinine as glomerular marker was dissolved, as well as 32 mg of mannitol. In some experiments, 1 to 10 mg of uric acid, dissolved in hot lithium carbonate (2), was also present. In other experiments, uric acid-2-^{14}C,

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prepared by Isotopes, Inc., of Westwood, N.J., with a specific activity in the range of 40 to 50 millicuries per millimole, was administered in amounts of 2 microcuries. The absolute amount of uric acid in this labelled material therefore was always infinitessimal, in the range of 0.008 mg.

In several studies, both unlabelled uric acid and uric acid-2-^{14}C were administered simultaneously into the renal artery. All intra-renal-arterial injections were given within 3 seconds. Urine samples were collected at successive 15 second intervals after intra-renal-arterial injection, for a total of 5 to 7 minutes.

In all experiments, an intravenous infusion of 5% mannitol in water was given at a rate of 6 ml per minute to maintain adequate urine flow. Since the normal urinary excretion level for uric acid in dogs, particularly in the mongrel, is extremely low (12), urinary excretion of injected unlabelled uric acid could be accurately measured with minimal blank correction in close-arterial experiments. When probenecid was used, it was given intravenously as a priming dose of 1.0 gm, then at a rate of 24 mg per minute intravenously as sustaining dose. An intra-renal-arterial injection experiment was carried out at least 30 minutes after injection of the priming dose of probenecid.

Analytical techniques for uric acid and creatinine have been previously described (13). ^{14}C-labelled uric acid was measured in urine samples in a Tri-Carb Liquid Scintillation Counter, using dioxane as the solvent. The purity of the infused ^{14}C-uric acid was assured by paper chromatography, as well as by the isotope-dilution technique.

The corrected percentage of the intra-renal-arterial injected substance excreted on the injected (experimental) side per urine specimen (w) was calculated as described by Chinard (11), as was the total corrected percentage of injected substance excreted (W). The value w, per urine specimen, expressed as a percentage, equalled the amount of substance excreted on the experimental side, minus the amount excreted on the control side, divided by the amount injected. The sum of w of all specimens in the experimental run equalled W, the total corrected percentage excreted.

Results

INTRA-RENAL-ARTERIAL INJECTION OF UNLABELLED URIC ACID

Fifteen experiments were done in female mongrel dogs. Figure 1 depicts a typical experiment. W for all such experiments appears in Table I. In individual experiments, W_{urate} showed a large range of variation, from 5.7 percent to 29.1 percent. There was a correspondingly wide range of variation in $W_{\text{creatinine}}$ (from 9.1 percent to 37.9 percent). The ratio $W_{\text{urate}}/W_{\text{creatinine}}$ for each experiment varied over a narrower range, from 0.61 to 1.16, with a mean of 0.88 ± 0.03.

One experiment made in a female Dalmatian dog showed a distinctly higher curve for uric acid than for creatinine, W_{urate} being 26.9 percent, $W_{\text{creatinine}}$ 18.8 percent, with $W_{\text{urate}}/W_{\text{creatinine}}$ 1.43 (Fig. 2(a)).

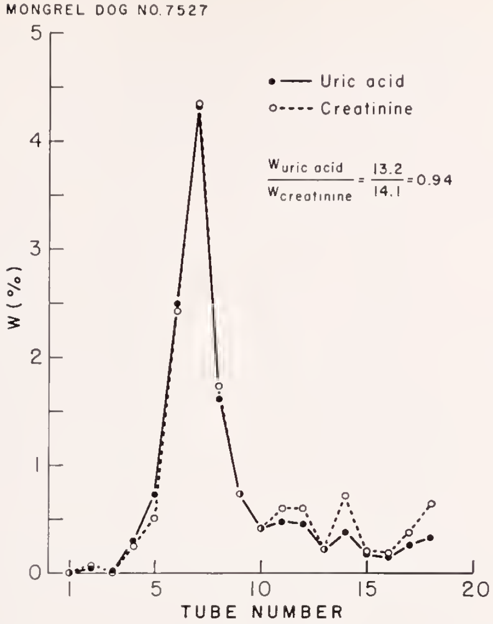

FIG. 1. Simultaneous intra-renal-arterial injection of uric acid and creatinine in a mongrel dog. In this study, total corrected percentage of uric acid excreted ($W_{\text{uric acid}}$) was 94 percent of corrected percentage of glomerular marker excreted ($W_{\text{creatinine}}$). In early urine specimens in this particular study there was precession of uric acid in relation to creatinine, suggesting early introduction of uric acid into the distal tubule by secretion. However, this was not consistently found in such experiments.

INTRA-RENAL-ARTERIAL INJECTION OF URIC ACID-2-^{14}C AND UNLABELLED URIC ACID

Seven experiments were done in which both uric acid-2-^{14}C and unlabelled uric acid were simultaneously injected into the renal artery, together with creatinine. Table II contains the values of W in all such experiments. W_{urate} was comparable to $W_{^{14}\text{C-urate}}$ ($W_{^{14}\text{C urate}}/W_{\text{urate}}$ varying from 0.81 to 1.26, with a mean of 1.00 ± 0.05), and the ratio of urate elimination to creatinine elimination was less than one (mean of 0.81 ± 0.05), with a practically identical value for $W_{^{14}\text{C-urate}}/W_{\text{creatinine}}$ (mean of 0.80 ± 0.04). In three additional experiments, variation of the load of uric acid injected into the renal artery (1 mg, 5 mg, and 10 mg) did not alter the ratio $W_{\text{urate}}/W_{\text{creatinine}}$.

EFFECT OF PROBENECID ON THE EXCRETION OF URIC ACID INJECTED INTO THE RENAL ARTERY

Four studies were done in mongrel dogs, in which uric acid-2-^{14}C and creatinine were injected into the renal artery before and after systemic administration of probenecid. No consistent effect of probenecid could be found in such studies (Table III). In two studies, $W_{^{14}\text{C-urate}}/W_{\text{creatinine}}$ rose slightly, in one the ratio fell somewhat, and in the fourth study there was essentially no change.

In a study done in one female Dalmatian dog, probenecid lowered $W_{^{14}\text{C-urate}}$ to

TABLE I

Values of W in Experiments in Which Unlabelled Urate and Creatinine Were Injected into Renal Artery

Experiments	W_{Urate} (%)	$W_{Creatinine}$ (%)	$W_{Urate}/W_{Creatinine}$
Mongrel Dogs			
1	21.9	24.3	0.90
2	17.5	15.1	1.16
3	26.4	29.3	0.90
4	17.1	18.0	0.95
5	16.3	17.9	0.91
6	19.9	21.1	0.94
7	13.2	14.1	0.94
8	11.2	12.1	0.93
9	29.1	37.9	0.77
10	19.0	19.7	0.96
11	5.7	9.3	0.61
12	5.7	9.1	0.63
13	24.8	26.5	0.94
14	28.1	30.1	0.93
15	9.3	11.6	0.83
			Mean: 0.88 (±SE) (±0.03)
Dalmatian Dog			
1	26.9	18.8	1.43

a value approaching $W_{creatinine}$, $W_{^{14}C\text{-urate}}/W_{creatinine}$ falling from 1.59 to 1.18 (Table III). In another Dalmatian dog, probenecid lowered W_{urate} to a value approaching $W_{creatinine}$, $W_{urate}/W_{creatinine}$ falling from 1.43 to 1.02 (Fig. 2).

Discussion

The experiments with unlabelled uric acid demonstrate that after intra-renal-arterial injection, uric acid excretion approaches excretion of a glomerular substance, creatinine, in the mongrel dog. Since urate excretion is known not to be handled solely by glomerular filtration, the similarity of the urate curve to the glomerular curve suggests the presence of simultaneous tubular processes of reabsorption and secretion in addition to glomerular filtration. In the mongrel dog, there is a relatively large renal tubular reabsorptive capacity for uric acid, as in man (1, 2, 3, 5), which tends to obscure the renal tubular secretory process. Only with heavy loading of uric acid, and marked inhibition of tubular reabsorption, can frank secretion be demonstrated in the mongrel dog, or in man. In the Dalmatian dog, on the other hand, in which renal tubular reabsorption of uric acid is deficient, frank uric acid secretion is more readily demonstrated. The possibility that the urate curve in the mongrel dog approaches the glomerular curve, due to the overwhelming load of unlabelled uric acid, causing saturation of renal

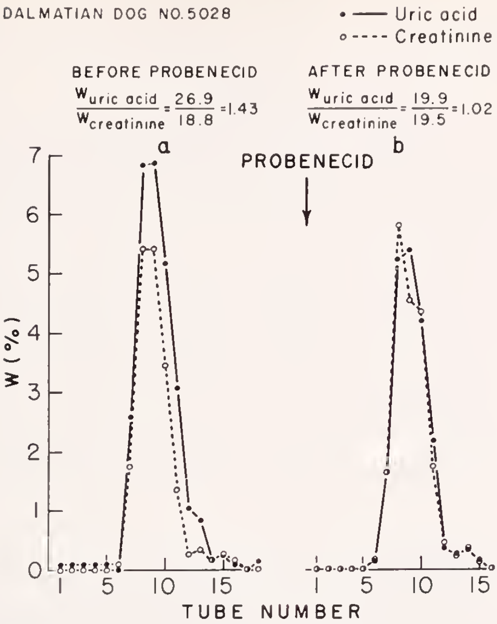

FIG. 2. Simultaneous intra-renal-arterial injection of uric acid and creatinine in a Dalmatian dog, before and after probenecid. Before probenecid, peak excretion per specimen for uric acid exceeds that for creatinine, and $W_{\text{uric acid}}$ exceeds $W_{\text{creatinine}}$ by 43 percent. There is a suggestion of minimal distal tubular secretion of uric acid, evidenced by slight excess of $W_{\text{uric acid}}$ over $W_{\text{creatinine}}$ in early tubes, presumably representing distal tubular fluid. The more significant tubes where $W_{\text{uric acid}}$ exceeds $W_{\text{creatinine}}$ are the peak tubes for each, and tubes subsequent to peak tubes, presumably more representative of proximal tubular fluid. After probenecid, $W_{\text{uric acid}}/W_{\text{creatinine}}$ falls from 1.43 to 1.02, and the curves for uric acid and creatinine approximate one another.

TABLE II

Values of W after Simultaneous Intra-Renal-Arterial Injection of ${}^{14}C$-Urate, Unlabelled Urate, and Creatinine in Mongrel Dogs

Experiments	$W_{^{14}\text{C-Urate}}$ (%)	W_{Urate} (%)	$W_{\text{Creatinine}}$ (%)	$W_{^{14}\text{C-Urate}}/W_{\text{Urate}}$	$W_{^{14}\text{C-Urate}}/W_{\text{Creatinine}}$	$W_{\text{Urate}}/W_{\text{Creatinine}}$
1	7.2	5.7	9.3	1.26	0.77	0.61
2	10.2	9.3	11.6	1.10	0.88	0.80
3	25.2	24.8	26.5	1.02	0.95	0.94
4	12.8	13.3	15.0	0.96	0.85	0.89
5	5.4	5.7	9.1	0.95	0.59	0.63
6	12.1	13.2	14.6	0.92	0.83	0.90
7	22.9	28.1	30.8	0.81	0.74	0.91
				Mean: 1.00	Mean: 0.80	Mean: 0.81
				(±SE) (±0.05)	(±SE) (±0.04)	(±SE) (±0.05)

tubular transport mechanisms, with resultant derivation of the major proportion of urinary urate from glomerular filtration, is excluded because the excretion curve for uric acid-2-${}^{14}C$ (containing 0.008 mg uric acid) without carrier was found to have the same relation to the glomerular curve as did the curve for carrier

TABLE III

Effect of Probenecid on the Ratio $W_{^{14}C\text{-}Urate}/W_{Creatinine}$

Experiments	Before Probenecid			After Probenecid		
	$W_{^{14}C\text{-}Urate}$ %	$W_{Creatinine}$ %	$W_{^{14}C\text{-}Urate}/W_{Creatinine}$	$W_{^{14}C\text{-}Urate}$ %	$W_{Creatinine}$ %	$W_{^{14}C\text{-}Urate}/W_{Creatinine}$
Mongrel Dogs						
1	13.4	25.9	0.52	12.3	22.1	0.55
2	8.4	16.1	0.52	11.4	16.7	0.68
3	8.8	12.3	0.71	10.5	11.9	0.88
4	14.3	20.0	0.72	16.8	29.7	0.57
Dalmatian Dog						
1	37.0	23.2	1.59	30.1	25.4	1.18

uric acid. Under other experimental circumstances, uric acid-2-^{14}C has been shown to exceed glomerular marker in the urine after intra-renal-arterial injection, a finding also taken to be compatible with renal tubular secretion of uric acid (10). In the studies reported by Zins and Weiner (14), and by Mudge and collaborators (15), excretion of uric acid in excess of that of glomerular marker has been demonstrated under conditions of modified stop-flow, with intra-renal-arterial injection of amounts of uric acid much larger than used in our experiments.

In the Dalmatian dog, intra-renal-arterial injection of uric acid produced a uric acid excretion significantly greater than that of glomerular marker. This clearly indicates renal secretion of uric acid in this species. Probenecid caused suppression of secretion, an effect previously well demonstrated in various species in which frank renal tubular secretion of urate exists (16, 17). After probenecid, however, $W_{\text{uric acid}}$ did not fall to a value less than that for glomerular marker; therefore, no clear evidence of renal tubular reabsorption of uric acid was obtained in these experiments. Some tubular reabsorption of urate in the Dalmatian has been previously shown, of lesser degree than that evident in mongrel dogs (1).

The failure of probenecid to elicit a marked effect on urate transport in the mongrel dog may be due to concomitant and proportional effects of probenecid on both tubular secretion and reabsorption in that species. Such effects have been described to account for the failure of intermediate doses of salicylate to alter urate excretion in man (18).

One further point to consider in the experiments presented here is the question of whether any significant degree of conversion of uric acid to allantoin occurred in the course of these acute experiments. In three experiments no excess allantoin excretion was found in any of the urine specimens collected over the 5 to 7 minute urine collection period of our studies. However, limitations in the method of allantoin analysis (19) do not exclude completely the possibility of conversion of small amounts of uric acid to allantoin after intra-renal-arterial uric acid injection.

Summary

The intra-renal-arterial injection technique of Chinard has been used to study the urinary excretion pattern of uric acid in mongrel and Dalmatian dogs. In mongrel dogs, uric acid excretion after instantaneous intra-renal-arterial injection is slightly less than that of a simultaneously injected substance handled by glomerular filtration alone, and is not influenced by probenecid administration. In the Dalmatian dog, uric acid excretion after intra-renal-arterial injection is greater than that of simultaneously injected glomerular substance, and is inhibited to the level of excretion of glomerular substance by probenecid. The data in the mongrel dog are compatible with the existence of simultaneously operating renal tubular processes of reabsorption and secretion, with reabsorption predominating. In the Dalmatian dog, the data are compatible with a preponderant renal tubular process of secretion.

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Renal Intracellular Phosphate and Phosphate Excretion: The Effect of Digoxin and Parathyroid Hormone*

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The capacity of the kidney to transport inorganic phosphate has been evaluated for the most part in terms of rate of clearance, filtration, reabsorption, and secretion. Since phosphorus plays such an important role in the intermediary metabolism of renal tubular cells, it seems clear that its rate of incorporation and subsequent release in connection with various metabolic processes and carrier transport systems may have an important impact on its rate of excretion. We have previously reported that cardiac glycoside infusion into one renal artery is followed by an ipsilateral increase in the rate of urinary excretion of phosphate, chiefly by depressing its tubular reabsorption by some 33 percent (1). That this phosphaturia is not related solely to the accompanying diuresis is suggested by the failure to observe changes in rate of phosphate excretion following administration of mercurial diuretics and chlorothiazide. Farnsworth (2) also did not find changes in phosphate excretion during diuresis induced by Mercupurin, but Wesson (3) noted a phosphaturia in 4 of 7 experiments with Mercurin. In addition, phosphaturia was not observed when diuresis was caused by osmotic agents such as urea (3–5) and mannitol (6), or during water diuresis (7).

Parathyroid hormone has long been known to cause increased urinary excretion of inorganic phosphate. Pullman et al (8) and Samiy et al (9, 10) concluded that the increase in urinary excretion of phosphate was a manifestation of a direct renal tubular action by parathyroid hormone. In the latter series of experiments, since no significant transtubular flux of systemically administered $^{32}PO_4$ was observed during the period of ureteral clamping, the authors concluded that there was no evidence that parathyroid hormone mediated a secretory process for phosphate excretion. However, prior work reported by Nicholson and Shepherd (11) and by Nicholson (12), in which various segments of the nephron were selectively damaged, suggested that phosphate was secreted by the distal tubule and that this process was enhanced by parathyroid hormone. While evidence for a transient secretion of phosphate was reported by Carrasquer and Brodsky (13) in response to instantaneous renal arterial injection of NaH_2PO_4, Handler (14) was unable to demonstrate net tubular secretion of inorganic phosphate over a wide range of plasma concentrations of inorganic phosphate.

To our knowledge, there are no reports in which a change in urinary excretion of inorganic phosphate has been correlated with the content and specific activity of ^{32}P of inorganic phosphate and selected phosphorylated

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organic compounds within renal tissue. In the present series of experiments, either digoxin or parathyroid hormone was administered into one renal artery of the dog. The rate of inorganic phosphate excretion from each kidney was monitored. The observed changes have been correlated with the content of various phosphorylated organic compounds and inorganic phosphate in perchloric acid extracts of renal cortical tissue obtained from each kidney at the conclusion of the experiment. Our data suggest that inorganic phosphate transport into renal cells is mediated by, or directly dependent on, its initial incorporation into several organic compounds, and that changes in net tubular reabsorption of phosphate are associated with alterations in intracellular content of inorganic phosphate.

Methods

Eleven experiments were carried out in which the effects of digoxin were studied. Six experiments were performed in which the effects of parathyroid extract were studied. In the latter series of studies, total parathyroidectomy was performed 2 to 10 days earlier.

Female mongrel dogs weighing 12 to 27 kg were anesthetized with sodium pentobarbital, 30 mg/kg. Five hundred to 1000 ml of isotonic sodium chloride solution, containing an additional 4 mEq/L potassium chloride, was administered intravenously following induction of anesthesia and during the course of the preparatory surgery. In the experiments in which parathyroid hormone was to be infused, additional phosphate, as NaH_2PO_4, was infused at the rate of 25 μM to 50 μM/minute. Both ureters were isolated and catheterized through a small suprapubic incision. This permitted simultaneous collection of timed urine samples from each kidney. The origin of the right renal artery was identified after a retroperitoneal dissection through the right flank. A curved hollow-bore 30 gauge needle attached to a long length of number 10 polyethylene tubing was then inserted into the origin of the right renal artery. Clotting in this needle was prevented by a continuous infusion of isotonic sodium chloride at 0.5 ml/minute.

A cannula with stylus was inserted into the carotid artery for blood sampling. A priming solution containing creatinine and para-aminohippurate for the estimation of glomerular filtration rate and renal plasma flow, respectively, was then administered intravenously, followed by another solution containing these substances at a rate designed to maintain the plasma concentration at nearly steady levels. After a suitable interval for equilibration, two or three premedication clearance periods were obtained simultaneously from each kidney for either 10 or 20 minutes depending on the urine flow. Without altering the rate of infusion into the renal artery, the isotonic saline solution was then replaced with one containing 0.05 mg/kg digoxin diluted in a volume of isotonic saline that was infused completely within 20 minutes. The isotonic sodium chloride solution was then restarted and continued until the end of the experiment. For the studies involving parathyroid hormone, 0.2 ml of a commercial parathyroid extract (Lilly) containing 100 units/ml was diluted in 90 ml of isotonic saline so that 0.11 units of the parathyroid extract was administered per minute. This solution was administered until the end of the experiment. Twenty minutes after the commencement of the intra-arterial infusion of either digoxin or parathyroid extract, a small quantity of radioactive phosphorus as $NaH_2{}^{32}PO_4$ was rapidly administered intravenously. The experiments were terminated about 120 minutes thereafter by quickly removing and plunging each kidney into liquid nitrogen. In three experiments in which digoxin was administered, the tracer dose of ^{32}P was administered intravenously at a steady rate so that the specific activity of the plasma was maintained nearly constant.

ANALYTIC METHODS

The samples of the plasma and urine from each kidney were analyzed for creatinine, para-aminohippurate and phosphate by methods routinely used in our laboratory (1).

In the experiments in which digoxin was studied, the kidney, while still frozen, was cracked into several fragments in a cold room at −5°C. Pieces of frozen cortex were weighed, pulverized, and directly extracted in cold perchloric acid as outlined by Hurlbert et al (15). In the more recent experiments in which parathyroid hormone was studied, each frozen kidney was fractured and large fragments were lyophilized. When dry, segments of renal cortex were easily identified, separated, and pulverized. The dry powder was added, after weighing, directly to cold perchloric acid, and extraction of the acid-soluble phosphate compounds was carried out as indicated above. The content of various compounds is expressed in terms of wet weight in the digoxin experiments, and dry weight in the parathyroid experiments.

An amount of extract equivalent to 0.25 to 1.0 gm of dry tissue was placed on Dowex 1 resin (Cal Biochem AG 1-×2 200–400 mesh (chloride form)) columns 22 cm long. The nucleotides and inorganic phosphate were eluted from the resin column at pH 2 by gradually increasing the chloride concentration of the eluate from 0.01 M to 0.55 M. Three pairs of nucleotides, DPN and AMP, GMP and UMP, and UDP and ATP did not resolve well at pH 2. Each of these pairs was further isolated by rechromatography at pH 4.5. The homogeneity of each nucleotide fraction was verified by phosphate analysis and the characteristic UV molar absorbency of each nucleotide at 250, 260, and 280 mμ.

To test the recovery of the nucleotides from this system, ten samples of solutions containing known quantities of different nucleotides, each simulating a perchloric acid extract of renal tissue, were prepared and chromatographed as outlined above. Recovery of the nucleotides as determined by UV absorption ranged from 92–98 percent, averaging 95 percent. However, some hydrolysis did occur to the extent that recovery of the monophosphates ranged from 110–118 percent, diphosphates from 90–95 percent, and triphosphates from 76–82 percent. To further test the reliability and reproducibility of the above methods, four rabbits were nephrectomized and kidneys immediately frozen in liquid nitrogen. Perchloric acid extracts were made and chromatography was carried out as described. The results compared favorably with those obtained by Gerlach, Bader, and Schwoerer (16) with paper chromatography. Additional confirmation insofar as ATP values were concerned was obtained from analysis of a number of the extracts by an enzymatic technique (Lowry) in use in another laboratory (17).

^{32}P content of plasma and urine samples, and each sample of effluent from the ion exchange column was determined in a 1 ml aliquot. The remaining portion of the sample was used for appropriate chemical quantitation. Specific activity was calculated as the ratio of cpm ^{32}P to μM P.

Using this procedure it was possible to measure the total quantity and specific activity of inorganic phosphate eluted from the column. An estimate of intracellular inorganic phosphate and its specific activity was calculated by subtracting from the value obtained for total quantity as described above a smaller quantity of extracellular inorganic phosphate of the same specific activity as that found in the last sample of plasma. The quantity of extracellular inorganic phosphate was estimated to be equal to the product of the volume of extracellular fluid of the kidney, which approximates 40 percent of its wet weight (18), and the concentration of inorganic phosphate in the terminal plasma sample. While the validity of these estimations might affect the derived quantitation of intracellular inorganic phosphate, the data for each kidney were similarly derived, so that any difference between the two kidneys may be attributed to the intrarenal arterial infusion of either digoxin or parathyroid extract. For example, to the extent that the absolute extracellular quantity of inorganic phosphate might be less than that estimated, the quantity and specific activity of the ^{32}P in the intracellular pool will be higher than the computed value. In no event can the values for quantity and specific activity of intracellular inorganic phosphate exceed those for inorganic phosphate recovered from the column. Our estimates of intracellular quantities for both content and specific activity may well be too high, mainly because hydrolysis of highly labelled triphosphate and diphosphate nucleotides occurs after the material has been placed in the columns. No attempt has been made to correct for this.

Results

DIGOXIN

In this series of 11 experiments in which digoxin was infused, the values for glomerular filtration rate, renal plasma flow, and the extent of the ipsilateral diuresis and phosphaturia were similar to those noted in a previous report of 20 similar experiments (1). In the present study, which included measurements of specific activity of phosphorus in the urine from each kidney, it was found that with the onset of ipsilateral diuresis and phosphaturia, the specific activity of phosphorus in the urine from the ipsilateral (experimental) kidney was less than that from the control kidney. The maximum difference in the two sides was noted 40–60 minutes after the unilateral administration of the digoxin, and in some experiments a difference was still evident at 120 minutes. The maximum difference in specific activity of phosphorus in simultaneously collected urines ranged from 8–46 percent. The mean difference between the two kidneys was 19.9 ± 9.6 percent ($p = 0.05$).

To be certain that the lower specific activity of the urinary phosphorus from the experimental kidney was not related to the rapid intravenous injection of the tracer dose of ^{32}P, a series of three experiments was performed in which the concentration of the plasma ^{32}P was maintained at nearly steady levels via a sustaining infusion. The specific activities of phosphorus in urine are shown in Table I. The observations were much the same as in those experiments in which the tracer was rapidly injected.

We had previously noted a small but apparently significant difference ($p = 0.02$) in the acid-soluble labile phosphate content of renal cortical tissue obtained from glycoside treated kidneys as compared to control kidneys (13.5 ± 2.7 μM P/gm wet weight versus 15.7 ± 3.4 μM P/gm wet weight). These data, and the presently observed difference in the specific activity of phosphorus in the urine from each kidney suggested a more detailed examination of the renal content of inorganic phosphate and acid-soluble organophosphate compounds. To this end the nucleotides and inorganic phosphate were extracted and isolated from perchloric acid homogenates, as described under methods. A typical chromatographic analysis performed at pH 2.0 is illustrated in Figure 1. The values of UV absorbence at 260 mμ and counts per minute/ml (^{32}P) for each 10 ml sample of effluent are shown. The major peaks include DPN-AMP (tubes 22–35), inorganic phosphate (tubes 48–58), GMP-UMP (tubes 60–67), ADP (tubes 68–74), UDP-ATP (tubes 85–91), and GTP (tubes 93–95).

The results of the analyses for nucleotides are shown in Table II. As indicated previously, these values have not been corrected for minor hydrolysis which attended the isolation procedures. This would account for the some-

TABLE I

Effect of Digoxin

Comparison of Specific Activity of Phosphorus in Plasma and Simultaneously Collected Urine Samples*

Experiment Number	Plasma	Urine from Control Kidney		Urine from Experimental Kidney	
	SA	SA	Volume ml/minute	SA	Volume ml/minute
204	2243	2156	1.0	1358	6.5
203	2685	2625	1.6	2382	4.2
202	2050	1967	1.1	1792	7.2

$* \frac{\text{cpm } 32\text{P/ml}}{\mu\text{M P/ml}}$.

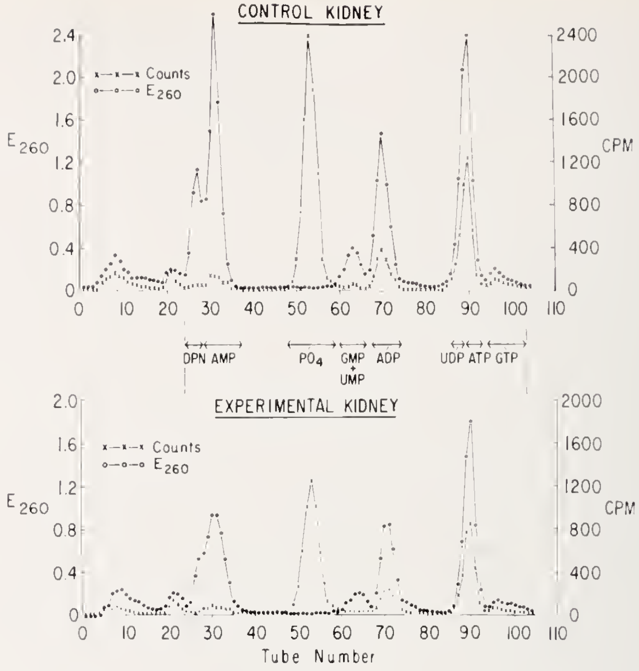

Fig. 1. Liquid chromatographic separation of various nucleotides and inorganic phosphate present in renal cortex.

TABLE II

Effect of Digoxin

Nucleotide Content μM/gm Wet Weight Renal Cortex

	Control Kidney	Experimental Kidney (Digoxin)
	n = 20 *	n = 11
ATP	0.88 ± 0.17†	0.83 ± 0.07
ADP	0.67 ± 0.12	0.59 ± 0.09
AMP	0.67 ± 0.14	0.63 ± 0.13
UDP	0.54 ± 0.13	0.55 ± 0.11
UMP	0.17 ± 0.08	0.16 ± 0.07
GTP	0.25 ± 0.08	0.28 ± 0.04
GMP	0.14 ± 0.05	0.10 ± 0.03
DPN	0.23 ± 0.05	0.27 ± 0.06
CMP	0.10 ± 0.03	0.11 ± 0.01

* nine additional control kidneys available from other experiments.

† standard deviation.

what lower values for triphosphates and higher values for monophosphates than have been found by Gerlach et al (16). Significant differences in the kidneys were not apparent, except for ADP ($p < 0.05$), GMP ($p = 0.025$), and possibly DPN ($p = 0.05$). Since the absolute values for the latter two substances were very much lower than for ADP, we are not satisfied, as yet, to accept the differences as indicative of any physiologic significance.

The values of the ratio of specific activity of radioactive phosphorus in each of the organo-phosphate compounds isolated to that of inorganic phosphate in the last plasma sample are listed in Table III. As can be seen, no significant differences in the kidneys were observed.

The highly variable content of inorganic phosphate in each kidney is listed in Table IV. As can be seen, 2 hours after the digoxin was infused into

TABLE III

Effect of Digoxin

Ratio of Specific Activity of Phosphorus in Nucleotides Isolated from Renal Cortex to that of Inorganic Phosphate in Final Sample of Plasma

$$\frac{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Renal Cortex)}}{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Plasma)}}$$

	Control Kidney	Experimental Kidney (Digoxin)
	n = 20*	n = 11
ATP	0.80 ± 0.12†	0.80 ± 0.07
ADP	0.59 ± 0.09	0.63 ± 0.07
AMP	0.31 ± 0.10	0.31 ± 0.08
UDP	0.64 ± 0.14	0.66 ± 0.13
UMP	0.36 ± 0.12	0.37 ± 0.12
GTP	0.58 ± 0.13	0.60 ± 0.14
DPN	0.10 ± 0.03	0.09 ± 0.02

* and † same as Table II.

TABLE IV

Effect of Digoxin

	Total Inorganic Phosphate μM/gm/wet wt		Intracellular Inorganic Phosphate μM/gm/wet wt		Specific Activity of $^{32}PO_4$ in Renal Cortex Relative to that of Last Plasma	
	Control	Experimental	Control	Experimental	Control	Experimental
	6.01	6.01	5.21	5.21	0.50	0.54
	3.43	3.14	2.66	2.37	0.67	0.68
	3.46	3.44	2.54	2.52	0.51	0.60
	3.80	3.02	3.08	2.30	0.45	0.58
	2.18	2.17	1.50	1.49	0.31	0.33
	6.66	5.84	6.05	5.23	0.75	0.72
	4.40	4.20	3.68	3.48	0.66	0.66
	11.60	10.00	10.90	9.56	0.71	0.72
	6.26	7.28	5.53	6.55	0.63	0.68
	9.18	9.02	8.51	8.35	0.60	0.64
	3.70	3.68	2.96	2.94	0.58	0.72
Mean	5.51	5.25	4.78	4.54	0.58	0.62
SD	2.82	2.59	2.85	2.67	0.13	0.11

one kidney, the mean values for each group did not differ. When results of each experiment are paired, the treated kidney was found to contain somewhat less inorganic phosphate than its control in nine experiments. In one experiment no difference was observed, and in another experiment the value was higher in the experimental kidney. This variability, for which we have no explanation, prohibits a conclusion that the mean of the paired differences is statistically significant ($p = 0.10$). It is conceivable that had we terminated the experiments somewhat earlier, perhaps at that time when the difference in the specific activity of the urinary phosphate was most pronounced, the present analytic methodology might have been sensitive enough to detect differences in renal content of inorganic phosphate. Listed in Table IV are paired data on specific activity of intrarenal inorganic phosphate which suggest that the experimental kidney contained a slightly more highly labelled inorganic phosphate pool ($p = 0.01$).

PARATHYROID HORMONE

Six experiments were completed in which parathyroid extract was infused into one renal artery of previously parathyroidectomized dogs. Renal hemodynamics, as indicated by creatinine and para-aminohippurate clearances, remained fairly stable throughout the course of each of these experiments. One hour or so following the start of the parathyroid hormone infusion, the urinary excretion of phosphate on the ipsilateral side was clearly greater than that from the control side. The cumulative excess in phosphate excretion from the ipsilateral kidneys amounted to 290–550 micromoles more than that from the control side.

Figure 2 illustrates the results of these experiments, the values being expressed as a mean of the ratio of the quantities from the experimental (parathyroid extract treated) and control kidneys. As can be seen, no significant variation from unity was noted in the creatinine clearances. In every clearance period after the start of the hormone infusion, the phosphate

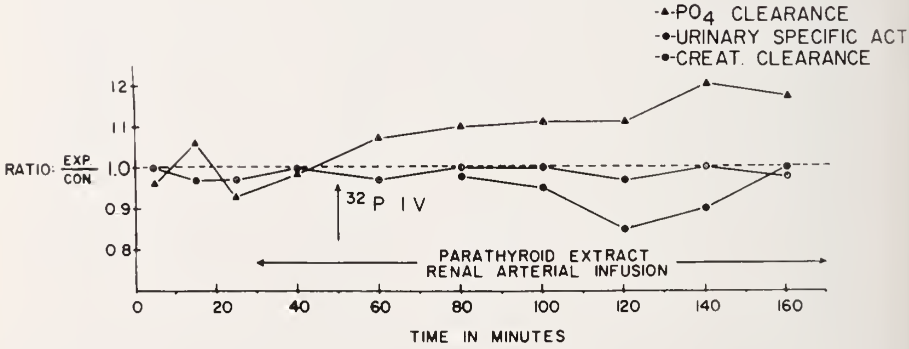

FIG. 2. Results in six experiments in which parathyroid extract was infused into one renal artery, expressed as a ratio of the values obtained in the experimental kidney to those in the control kidney.

TABLE V

Effect of Parathyroid Hormone
Nucleotide Content μM/gm Dry Weight Renal Cortex

	Control Kidney	Experimental Kidney (Parathyroid Hormone)
	n = 6	n = 6
ATP	6.24 ± 1.74*	6.23 ± 1.86
ADP	4.59 ± 0.57	4.33 ± 0.32
AMP	3.88 ± 0.83	4.25 ± 0.73
UDP	3.48 ± 0.87	3.39 ± 0.79
UMP	0.94 ± 0.28	0.90 ± 0.30
GTP	1.85 ± 0.40	1.40 ± 0.20
GMP	0.56 ± 0.27	0.64 ± 0.21
DPN	1.59 ± 0.46	1.64 ± 0.66
CMP	0.50 ± 0.19	0.50 ± 0.13

* standard deviation.

clearance by the experimental kidney ranged from 9–20 percent greater than by the control kidney. In sharp contrast, the ratio of the specific activities of phosphorus in the urine from both sides (experimental/control) progressively decreased for about 90 minutes after the commencement of the hormone administration. The maximum difference between the means was statistically significant ($p = 0.05$). Thereafter, there appeared to be a stepwise increase in the ratio of specific activities, so that by the end of the experiments, some 130 minutes after the start of the hormone, the difference in each kidney was no longer evident.

Table V shows the content of various organo-phosphate compounds which were isolated and identified in extracts of renal cortex. No significant differences were noted except for GTP ($p = 0.05$). The values for specific activity of phosphorus in these compounds are listed in Table VI. As can be seen, the mean value for specific activity of ATP in the experimental kidneys appeared to be somewhat higher than that for the control kidneys ($p = 0.1$). In Table VII, however, we have listed all the individual values of specific activity for each of the more highly labelled nucleotides. Only the means of the paired differences for ATP and ADP are statistically significant ($p = 0.0025$ and 0.05, respectively).

In respect to the inorganic phosphate content of renal cortex, and its specific activity, the values obtained in each experiment are recorded in Table VIII. While the difference in the mean values is not significant, the mean of the paired differences for inorganic phosphate content borders on significance ($0.05 < p < 0.1$). The mean of the paired differences in specific activity suggests that the observed increased specific activity in the experimental kidney is significant ($p = 0.005$).

Of particular concern, however, is the rather higher value and greater variability in the inorganic phosphate levels found in this series of experi-

TABLE VI

Effect of Parathyroid Hormone

Ratio of Specific Activity of Phosphorus in Nucleotides Isolated from Renal Cortex to that of Inorganic Phosphate in Final Sample of Plasma

$$\frac{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Renal Cortex)}}{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Plasma)}}$$

	Control Kidney	Experimental Kidney (Parathyroid Hormone)
	n = 6	n = 6
ATP	0.78 ± 0.06*	0.83 ± 0.06
ADP	0.53 ± 0.05	0.56 ± 0.06
AMP	0.31 ± 0.10	0.30 ± 0.11
UDP	0.59 ± 0.13	0.58 ± 0.10
UMP	0.45 ± 0.12	0.48 ± 0.15
GTP†	0.56 ± 0.11	0.58 ± 0.13
DPN	0.11 ± 0.04	0.11 ± 0.04

* standard deviation.
† n=5 (laboratory accident).

TABLE VII

Effect of Parathyroid Hormone

Ratio of Specific Activity of Phosphorus in Nucleotides Isolated from Renal Cortex to that of Inorganic Phosphate in Final Sample of Plasma

$$\frac{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Renal Cortex)}}{\text{cpm } {}^{32}\text{P}/\mu\text{M P (Plasma)}}$$

ATP		ADP		UDP		GTP	
Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
84.8	78.7	53.0	50.8	72.6	71.0	75.9	71.0
87.5	82.1	55.3	56.4	54.8	54.8	65.1	64.2
74.4	71.5	48.8	46.5	53.4	55.0	41.8	41.0
76.8	70.3	55.5	48.1	58.3	57.4	—	—
89.4	84.2	57.8	57.8	65.7	75.0	47.3	50.0
83.3	83.3	66.6	59.6	40.3	36.8	62.2	59.6
Mean 83.0	78.3	55.8	52.6	58.4	58.9	57.8	56.2
SD 5.5	5.5	5.5	5.2	10.3	12.5	12.5	10.8

ments in contrast to those observed in the series in which digoxin was infused. We believe that one factor may be primarily responsible for this difference. In the experiments in which parathyroid extract was injected, a sustaining infusion of phosphate at 25–50 μM/minute was administered. Plasma inorganic phosphate concentrations seemed to be similar in the two series, as did the filtered loads. However, the control urinary excretion rates in the parathyroidectomized animals were generally higher than in the intact animals in which digoxin was studied. Therefore, we ascribe the observed dif-

TABLE VIII

Effect of Parathyroid Hormone

	Total Inorganic Phosphate μM/gm/dry wt		Intracellular Inorganic Phosphate μM/gm/dry wt		Specific Activity of $^{32}PO_4$ in Renal Cortex Relative to that of Last Plasma	
	Control	Experimental	Control	Experimental	Control	Experimental
	38.6	39.9	35.4	36.7	0.61	0.63
	37.4	47.0	31.7	41.0	0.73	0.77
	60.0	56.2	53.2	48.0	0.71	0.74
	88.4	93.6	82.1	87.4	0.62	0.65
	44.3	50.1	38.7	45.5	0.81	0.81
	87.2	87.2	75.6	75.6	0.55	0.57
Mean	59.4	62.3	52.8	55.7	0.67	0.69
SD	23.4	22.0	21.6	20.7	0.09	0.09

ferences in the urinary excretion rates and renal contents of inorganic phosphate between the two series to the phosphate containing sustaining infusions in the parathyroid group.

Discussion

The striking observation in the present studies with both digoxin and parathyroid hormone is the lower specific activity of phosphate in the urine from the experimental kidney as its phosphate clearance increased. In the absence of differences in renal hemodynamics, this change in specific activity of phosphorus in the urine indicates that some portion of the increased urinary inorganic phosphate following the renal arterial infusion of either digoxin or parathyroid extract must have derived from intracellular loci. At the conclusion of each experiment, some 2 hours or so after starting the infusion of either digoxin or parathyroid extract, this difference between the kidneys was much less apparent in the digoxin studies, and no longer apparent in the parathyroid extract studies. It was at this time that the kidneys were removed for tissue analyses. We believe that the results of these analyses must be interpreted in the light of the preceding findings in the urine from each kidney.

With this in mind, in respect to the effect of digoxin, the major difference in nucleotide composition of the kidneys was the reduction in content of ADP in the experimental kidney. No alterations in the labelling of the nucleotides were observed. It is clear that a difference in the inorganic phosphate content (Table IV) of the experimental kidneys cannot be detected because of scatter in the results. Additional experiments must be done to determine if the small difference, if it exists at 2 hours or earlier, is significant. On the other hand, the specific activity of the inorganic phosphate pool in the experimental kidney is greater than in the control.

Since the urinary specific activity measurements indicate the loss of phosphate from renal cells, we believe that the results of the tissue analyses can be appropriately interpreted to support the view that there may well have been an early diminution in the size of the intracellular inorganic pool. This early response is then followed by increased turnover and restoration of the pool. The failure to observe any change in phosphate turnover within the nucleotides suggests that increased net incorporation and/or exchange of inorganic phosphate took place primarily across the peritubular membrane of renal cells. This argument follows from the consideration that there is net reduction in reabsorption of phosphate at the cell luminal surface.

An alternative proposal is that digoxin has no effect on phosphate transport or metabolism in those portions of the nephron within the renal cortex, but rather provokes a similar chain of responses from the medullary and/or papillary segments of the nephron. Both of these speculations can be subjected to further experimental analysis. We believe that the bulk of the evidence relating to the direct renal action of cardiac glycosides indicates that they inhibit ion transport via a membrane ATPase throughout the nephron. Recent studies by Allen et al (19) and Hendler et al (20) have shown that the activity of Na-K-ATPase is greater in the renal medulla than in the cortex. These investigators also have demonstrated that both digoxin (19) or ouabain (20) reduce the activity of the enzyme isolated from the renal medulla much more than the enzyme obtained from the cortex.

In respect to the effect of parathyroid hormone on nucleotide content, only the already small values for GTP seemed to be further reduced in the experimental kidney. The observed increment in specific activity of phosphorus in both ATP and ADP in the experimental kidney (Table VII) do appear to be significant. These findings are in contrast to those of De Verdier (21), who reported that ^{32}P incorporation into some ester fractions, particularly uridine compounds, was enhanced by parathyroid hormone. In his study, rats were exsanguinated 1.5 hours after parathyroid hormone had been injected. The kidneys were then removed and frozen. No ATP was isolated.

While the difference in the mean values for the cortical content of inorganic phosphate (Table VIII) following parathyroid extract infusion does not appear to be statistically significant ($p = 0.25$), paired differences in 4 of the 6 experiments suggest that the inorganic phosphate content within the renal cortex of the treated kidney was higher than the control kidney. Additional experiments will be required to sustain this conclusion.

The observations (Table VIII) in respect to specific activity of $^{32}PO_4$ in renal cortex support the view that parathyroid hormone did enhance the net incorporation of ^{32}P into inorganic phosphate. Similar observations have been reported by Egawa and Neuman (22). Since we observed that urinary specific activity was lower on the experimental side, we conclude that some of the inorganic phosphate which appeared in the urine shortly after starting the infusion of parathyroid extract derived from intracellular loci. Two hours or so later net incorporation of ^{32}P into the inorganic phosphate pool was increased in the treated kidney, therefore it is reasonable to conclude that the rate of turnover of intracellular inorganic phosphate must have been accelerated. It is conceivable, too, that changes in both content and specific activity of inorganic phosphate within the treated kidney might be more evident in the medullary portion of the kidney rather than in the cortex which was studied. In any event, since there is an increased loss of inorganic phosphate into the urinary space, it is logical to depict one site of this accelerated inorganic phosphate turnover to be at the peritubular membrane of the renal cell.

This view is consistent with the observation, by Chase and Aurbach (23, 24), that parathyroid hormone activates a membrane bound adenyl-cyclase and increases the production of cyclic AMP. These authors suggested that the phosphaturic effect is secondary to increased intracellular 3′5′ AMP, which itself might activate yet another renal cellular enzyme. This enzyme would, in effect, catalyze a series of reactions leading to the loss of phosphate from the cell. These authors also observed that vasopressin, an activator of adenyl-cyclase in tubular cells located considerably more distally, likewise produced a phosphaturia. Our data suggest that parathyroid hormone did not cause a net loss of phosphate from cortical cells, but rather either no net change or a small increase. That parathyroid hormone may induce accumulation of phosphate in renal cells is suggested by the observation by Rasmussen and DeLuca (25) that mitochondria, *in vitro*, did so in the presence of the hormone. These authors speculated that the parathryoid hormone accelerates the movement of phosphate ions across subcellular and cellular membranes. More recent work by Rasmussen et al (26) lends additional support to this speculation, but some doubt is expressed by Cohn et al (27), who found no specific *in vivo* effect of parathyroid hormone on renal mitochondria.

We believe that the acceleration of certain aspects of phosphate metabolism by parathyroid hormone, such as the conversion of ATP to cyclic AMP, leads to enhanced turnover of ATP and possibly ADP, and results in a more rapid turnover and some increase in the inorganic phosphate content of the cell and/or cell membrane. This phenomenon, in turn, causes a reduction primarily in net tubular reabsorption of inorganic phosphate. While it is not possible to rule out stimulation by parathyroid hormone of a tubular secretory process for inorganic phosphate, or even augmented exchange-diffusion, consideration of the quantity of inorganic phosphate in the urine and the lesser labelling of the intracellular inorganic phosphate pool relative to that of the plasma and urine makes these alternative explanations less attractive.

Foulks and Perry (28) suggested that alterations in renal cellular phosphate content may lead to changes in its tubular reabsorption. Eisenberg (29) postulated that parathyroidectomy should decrease renal medullary phosphate content. He also suggested that increased transport of calcium by renal tubular cells mediated by parathyroid hormone may be responsible for the associated increase in phosphate uptake and content in the kidney.

A final point that we believe is worthy of comment is the significance of the lesser labelling of the intracellular inorganic phosphate pool than that of either the terminal two phosphates in ATP and the terminal phosphate in ADP and UDP. Considering all data from all experiments, irrespective of whether digoxin or parathyroid hormone was infused, the labelling of the intracellular inorganic phosphate pool relative to that of the last plasma sample averaged about 65 percent. The specific activities of phosphorus in AMP and UMP averaged about 40 percent. This being the case, it follows that the specific activity of the terminal phosphates of UDP and ADP relative to that of the last sample of plasma averaged 85 percent and nearly 100 percent, respectively. Similarly, since the specific activity of ^{32}P in ATP was 80 percent that of the plasma inorganic phosphate, the two terminal phosphates must also have been more completely equilibrated with the inorganic phosphate in intravascular and extracellular fluids. Evaluation of the phosphate labelling in GTP also indicates that its terminal phosphates equilibrated more rapidly with that of plasma inorganic phosphate than did the intracellular pool of inorganic phosphate. These observations suggest, therefore, that the extracellular phosphate pool is probably the major source of phosphate which is incorporated into disphosphate and triphosphate nucleotides in the kidney. Alternatively, there may be multiple intracellular pools of inorganic phosphate, one or more located in the membrane and in more rapid equilibrium with extracellular inorganic phosphate.

Summary

Digoxin enhances the renal excretion of inorganic phosphate by directly inhibiting its tubular reabsorption as well as promoting its loss from renal tubular cells. Except for a reduction in ADP content, no significant differences in the renal content of several nucleotides was noted.

Parathyroid hormone directly augments the efflux of phosphate from intracellular loci accounting in part for the observed diminished net tubular reabsorption. In addition, labelling of ATP and ADP is increased, as is that of the inorganic phosphate pool. While the present limited series of experiments do not conclusively demonstrate an absolute increase in renal content of inorganic phosphate following the infusion of parathyroid extract, we believe that alterations involving metabolism of phosphate lead to a small increment in the content of inorganic phosphate within the renal cell and/or its membrane which reduces net tubular reabsorption of phosphate.

Our data suggest that inorganic phosphate transport into renal cells is primarily mediated by its initial incorporation into one or more diphosphate and triphosphate nucleotides.

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The Urinary Diluting and Concentrating Operation in Caval Dogs*

JEROME G. PORUSH, M.D.

Chronic constriction of the thoracic portion of the inferior vena cava of the dog leads to salt and water retention and the accumulation of edema and ascitic fluid (caval dogs). This model has been extensively used for the study of sodium retention, particularly by Davis and his associates (1–4). In general, most of the reports dealing with the caval dog have attempted to elucidate the factors leading to the altered excretion of sodium. It now seems clear that sodium retention occurs in these animals in the absence of a depression in glomerular filtration rate (GFR) and filtered sodium, and that hyperaldosteronism is a contributory but not necessary factor (4). It has been demonstrated that the characteristic decrease in tubular sodium reabsorption following saline loading is either absent or reduced in magnitude in caval dogs (5–7). Whether this phenomenon is related to an alteration in the reflex arc associated with a natriuretic hormone or in intrarenal hemodynamics is not entirely known and must await further clarification of all the factors which control renal sodium excretion in normal animals.

Knowledge of the tubular site of increased sodium reabsorption in caval dogs may help to elucidate the mechanism of sodium retention in these animals. This information, in turn, may be useful in defining the factors which control sodium excretion under physiological conditions. Micropuncture experiments of Cirksena, Dirks, and Berliner (6) suggest that sodium retention in dogs with acute constriction of the thoracic inferior vena cava is mediated at least in part by an increase in fractional sodium reabsorption along the proximal tubule. In previous experiments from our laboratory it was demonstrated that caval dogs had a greater natriuretic response to angiotensin II than normal dogs, and that the inhibition of sodium reabsorption by angiotensin appeared to take place in the proximal tubule as well as the ascending limb of Henle's loop (8). It was also noted that the concentrating and diluting operations of the kidney differed in the caval dog in such a manner as to suggest that there was increased sodium reabsorption at other sites of the nephron in addition to the proximal tubule. The possibility that the greater natriuretic effect of angiotensin in caval dogs is related to sodium retention prompted us to examine the urinary concentrating and diluting operation in these animals more thoroughly in an attempt to localize the tubular sites of increased sodium reabsorption.

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Brief Description of Methods

In all the experiments to be reported, classical clearance methodology was employed. Production of chronic caval dogs, preparation of the dogs for hydrated or hydropenic experiments, methods of infusion, and calculation of clearances have been described previously (8). Solute free water clearance (C_{H_2O}) was calculated as V-C_{osm} where V = urine flow in ml/minute, and C_{osm} = solute clearance. Solute free water reabsorption ($T^c_{H_2O}$), an indirect measure of urine concentration, is that amount of water which is removed from isotonic tubular fluid to concentrate the urine, and is calculated as C_{osm}-V. In those experiments in which para-aminohippurate extraction ratio (E_{PAH}) was determined, the left renal vein was catheterized and the extraction ratio calculated in the usual manner (9). The non-extracted portion of PAH was taken to represent non-cortical blood flow, part of which is presumed to be medullary blood flow (10–12). Tissue analysis for solute content was performed in some of the hydropenia experiments under either non-diuretic conditions or at various levels of 2% saline diuresis. In these experiments, the kidneys were rapidly removed and frozen in an acetone-dry ice mixture. Tissue was taken from the papillary tip (inner half of the inner medulla) and analyzed for sodium, potassium, ammonia, and urea as described by Goldberg and Ramirez (13). The urine collected prior to sacrificing the animal was kept under oil and analyzed for the same solutes. The osmolality or "sum of osmoles" of the papillary tip was calculated by adding the urea concentration to two times the sum of the concentration of sodium, potassium, and ammonia, in mmoles/kg H_2O. The final urine osmolality was calculated in a similar manner, which closely approximated the osmolality measured directly by freezing point depression. In all the results described below the clearances are given after factoring by GFR, and thus represent the clearance per 100 ml GFR.

Results and Discussion

In the first group of experiments the pattern of C_{H_2O} and sodium clearance (C_{Na}) during water diuresis or hypotonic saline diuresis was compared in normal and caval dogs. The experiments during water diuresis are depicted in Figure 1, where it is evident that C_{H_2O} was similar in normal and caval dogs. However, because of the lower C_{Na} in caval dogs, distal sodium load, as calculated by $C_{H_2O} + C_{Na}$, was significantly lower in these animals ($p < 0.05$). In order to clear free water at the same rate as the normal dog, but with a lower level of supply of sodium to the water clearing segment of the nephron, the caval dog must reabsorb a larger fraction of the sodium presented to this site. The formula $C_{H_2O}/(C_{H_2O} + C_{Na})$ may be used as an index of fractional sodium reabsorption in the water clearing segment. Accordingly, 93.0 percent of the sodium reaching this site was reabsorbed in the caval dog compared to 83.0 percent in normal dogs ($p < 0.05$).

During hypotonic saline diuresis, the same pattern of increased sodium reabsorption in the water clearing segment was noted in caval dogs (Fig. 2). The difference between normal and caval dogs was more dramatic than during water diuresis, as fractional sodium reabsorption in the water clearing site was 79.8 percent in the caval dog and only 49.1 percent in normal animals ($p < 0.01$). The pattern of distal tubule sodium reabsorption in caval

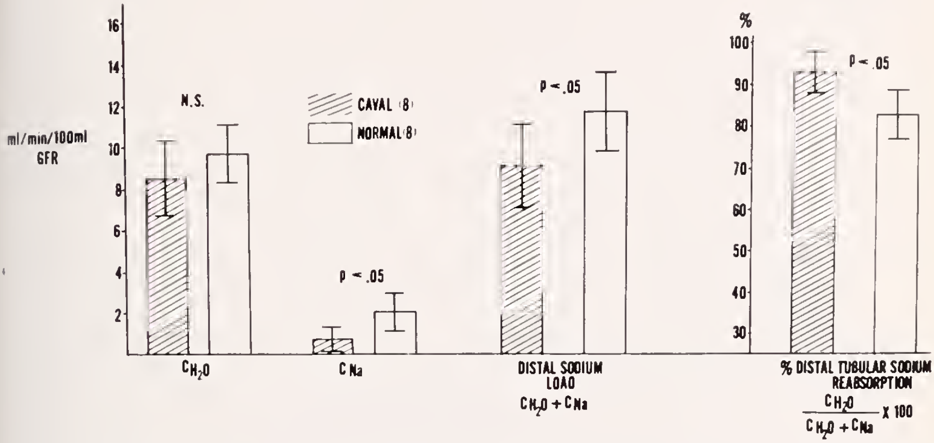

FIG. 1. Fractional free water clearance (C_{H_2O}), sodium clearance (C_{Na}), distal sodium load ($C_{H_2O} + C_{Na}$), and percent sodium reabsorption in the distal tubule ($C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$) at the period of peak free water clearance during water diuresis. The hatched column represents the mean ± 1 SD in eight caval dogs, and the open column the mean ± 1 SD in eight normal dogs.

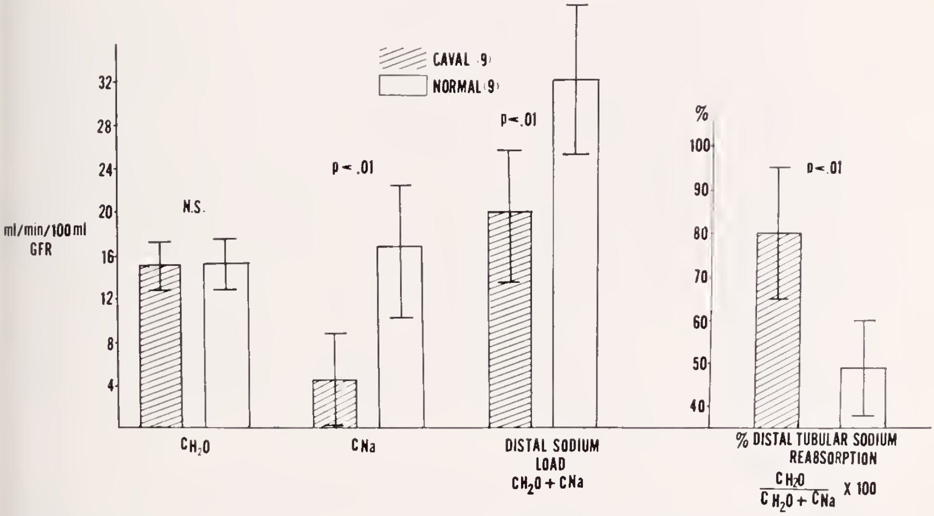

FIG. 2. Fractional free water clearance (C_{H_2O}), sodium clearance (C_{Na}), distal sodium load ($C_{H_2O} + C_{Na}$), and percent sodium reabsorption in the distal tubule ($C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$) at the period of peak free water clearance during hypotonic NaCl diuresis. The hatched column represents the mean ± 1 SD in nine caval dogs, and the open column the mean ± 1 SD in nine normal dogs.

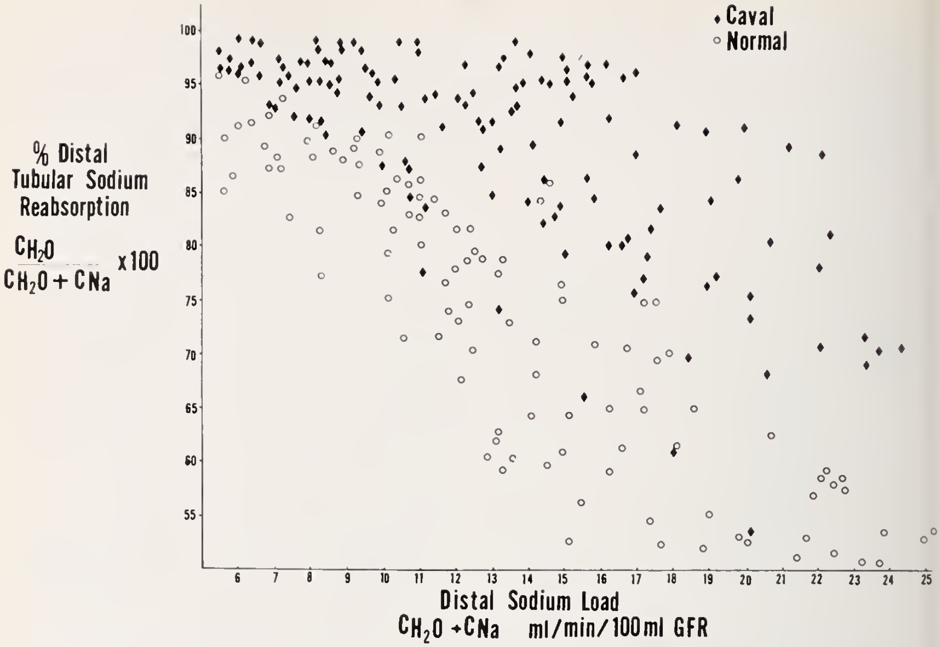

FIG. 3. Relationship between percent distal tubular sodium reabsorption ($C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$) and fractional distal sodium load ($C_{H_2O} + C_{Na}$ 100 ml GFR) during hypotonic NaCl diuresis. The solid diamonds represent all the clearance periods from nine experiments in caval dogs, and the open circles represent all the clearance periods from nine experiments in normal dogs.

and normal dogs is illustrated in Figure 3. It is evident that at every level of distal sodium load percent sodium reabsorption was greater in the caval dog, and at higher rates of distal sodium delivery the decrease in fractional sodium reabsorption was much less in these animals.

Since the level of distal sodium supply represents that moiety of sodium which escapes reabsorption in the proximal tubule and descending limb of Henle's loop, it provides an index of proximal tubule sodium reabsorption. Thus, 81.5 percent of the filtered sodium was reabsorbed proximal to the ascending limb in caval dogs, and only 68.0 percent in normal dogs ($p < 0.01$) during hypotonic saline diuresis. The greater percent of proximal tubule sodium reabsorption in the caval dog was observed despite an equal or greater volume of hypotonic saline infused, and a similar degree of extracellular volume expansion as estimated by the decrease in serum sodium concentration in each group.

In another group of experiments hypotonic mannitol was infused to determine whether the pattern of increased sodium reabsorption in the ascending limb would be seen in caval dogs when sodium supply to this site was increased by an osmotic diuretic as well as by extracellular volume expansion. Moreover, since a limiting gradient for sodium reabsorption is achieved in the proximal tubule with mannitol (14), it was also possible to test the capacity of both groups of animals to reabsorb sodium in the ascending limb against a less favorable gradient. Figure 4 demonstrates the findings during hypotonic mannitol infusion and, again, the capacity to reabsorb sodium in the water clearing segment was significantly greater in caval animals, 86.4 percent compared to 64.8 percent in normal dogs ($p < 0.01$). The increased sodium reabsorption at this site was noted in caval dogs despite the lower sodium concentration in the tubular fluid as approximated by $(C_{Na} + C_{H_2O})/V$. In the caval dog, this ratio was 0.59 compared to 0.73 in normal dogs ($p < 0.05$).

The above experiments clearly demonstrate that the caval dog can clear free water at a rate at least as great as normal dogs, but at a significantly lower level of sodium supply to the water clearing segment. Furthermore, the increased capacity to excrete free water was evident even when the gradient for sodium reabsorption was made less favorable by the infusion of mannitol. Insofar as the clearance of free water is dependent primarily upon sodium reabsorption in the diluting segment of the nephron (15–18), it follows that fractional sodium reabsorption is increased at this site in the caval dog. In addition, these experiments demonstrate that sodium reabsorption is increased in the proximal tubule in caval dogs since distal sodium supply was significantly lower in caval dogs during all three diuretic states. Thus, the increase in fractional sodium reabsorption in the proximal tubule demonstrated by Cirksena et al (6) is not a transient phenomenon associated with acute constriction of the thoracic inferior vena cava, but

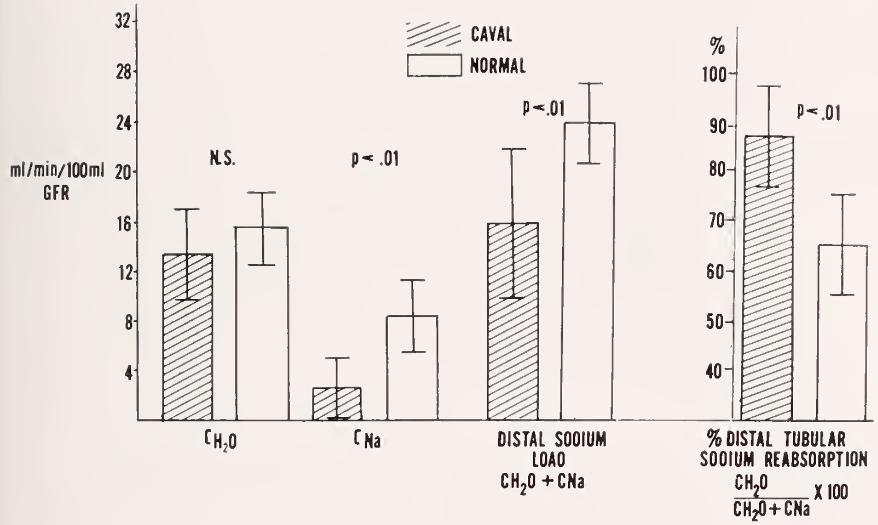

FIG. 4. Fractional free water clearance (C_{H_2O}), sodium clearance (C_{Na}), distal sodium load ($C_{H_2O} + C_{Na}$), and percent sodium reabsorption in the distal tubule ($C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$) at the period of peak free water clearance during hypotonic mannitol diuresis. The hatched column represents the mean ± 1 SD in five caval dogs, and the open column the mean ± 1 SD in five normal dogs.

persists in the chronic preparation as well, as suggested by Levinsky and Lalone (5) and Friedler, et al (7).

In the next group of experiments the concentrating operation of the kidney in normal and caval dogs was examined under non-diuretic conditions and during 2% saline or 10% mannitol diuresis during maximum hydropenia. Analysis of renal tissue solute content was carried out under non-diuretic conditions and at various levels of diuresis produced by 2% saline infusion. This enabled us to compare the osmolality of the papillary tip to the osmolality of urine collected immediately prior to sacrificing the animal. In most of these experiments the extraction of PAH was also measured.

The maximum urine concentration was similar in normal and caval dogs, 1780 and 1719 mOsm per kg H_2O, respectively. In Figure 5, $T^c_{H_2O}$ is compared in normal and caval dogs as sodium clearance increased during 2% saline infusion. It is clear that $T^c_{H_2O}$ is higher in normal dogs, with a mean peak of 7.9 ml/min/100 ml GFR in these animals compared to 5.9 ml/min/100 ml GFR in caval dogs ($p < 0.01$). Not only was the peak level of $T^c_{H_2O}$ lower in caval dogs, but it occurred at much lower levels of sodium clearance (approximately 15 ml/min/100 ml GFR), with a striking tendency to decrease as sodium clearance increased further. In contrast, $T^c_{H_2O}$ did not reach a plateau in normal dogs until C_{Na} was approximately 30 ml/min/100 ml GFR, and a fall off was barely evident.

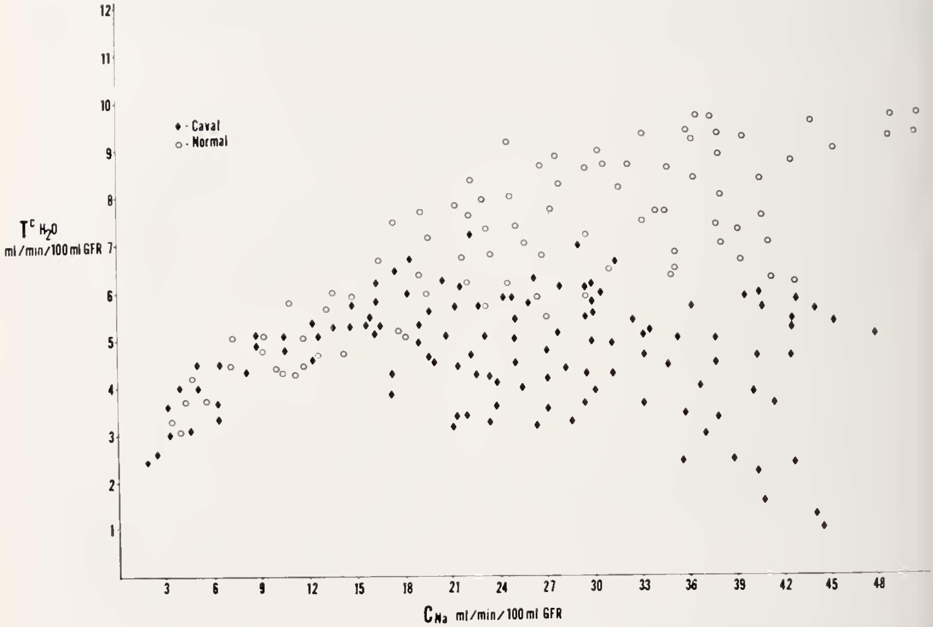

FIG. 5. The relationship between solute free water reabsorption ($T^c_{H_2O}$) and sodium clearance (C_{Na}) during 2% NaCl infusion. The solid diamonds and open circles represent all the clearance periods obtained from seven experiments in caval dogs and six experiments in normal dogs, respectively.

Figure 6 describes the same relationships during 10% mannitol infusion in normal and caval dogs. In general, the findings are similar to the saline experiments, with peak $T^c_{H_2O}$ significantly greater in normal dogs, 8.9 compared to 6.7 ml/min/100 ml GFR in caval dogs ($p < 0.01$). Again, $T^c_{H_2O}$ leveled off earlier in caval dogs with a greater tendency to decrease as sodium excretion increased beyond 15 ml/min/100 ml GFR. It is of interest that peak $T^c_{H_2O}$ was greater during mannitol than saline diuresis in each group, although the difference was statistically significant in caval dogs only (6.7 during mannitol diuresis and 5.9 ml/min/100 ml GFR during saline diuresis ($p < 0.05$). Moreover, in caval dogs the decrease in $T^c_{H_2O}$ after the peak level was reached was not as great during mannitol diuresis compared to saline diuresis.

Interpretation of these results is dependent upon several factors. Although urine concentration and $T^c_{H_2O}$ are probably most affected by the amount of sodium reabsorbed in the medullary portion of the ascending limb of Henle's loop (functioning as a countercurrent multiplier), this relationship is much more complicated than with C_{H_2O}. In addition, there are other factors affecting the level of $T^c_{H_2O}$ such as the degree of water permeability of the collecting duct, the rate of flow in the vasa recta, and the accumulation of urea in the tubular fluid and medullary interstitium.

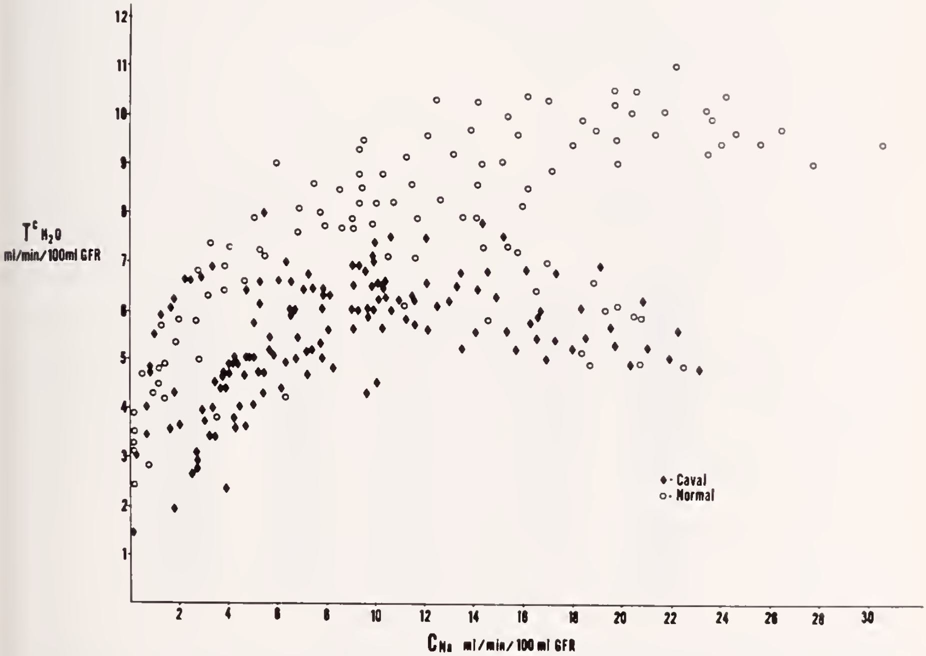

FIG. 6. The relationship between solute free water reabsorption ($T^c_{H_2O}$) and sodium clearance (C_{Na}) during 10% mannitol infusion. The solid diamonds and open circles represent all the clearance periods obtained from eight experiments in caval dogs and six experiments in normal dogs, respectively.

It is unlikely that the lower peak $T^c_{H_2O}$ in caval dogs reflects a decrease in sodium reabsorption in the ascending limb, since the experiments during water diuresis indicate increased sodium transport at this site. Although an increase in C_{H_2O} may reflect increased sodium reabsorption in the cortical portion of the ascending limb only (19), the magnitude of fractional reabsorption of sodium in the distal tubule of the caval dog, 79.8 percent during hypotonic saline diuresis and 86.4 percent during hypotonic mannitol diuresis (Figs. 2 and 4), strongly suggests that the medullary portion of the ascending limb is participating in the increased sodium reabsorption.

E_{PAH} was similar in caval and normal dogs under non-diuretic conditions, suggesting that non-cortical blood flow was the same in both groups (Fig. 7). During 2% saline diuresis the extraction ratio tended to decrease in both groups, with a greater fall noted in caval dogs (Fig. 8). However, when the data were grouped into successive 5 ml/minute increments of sodium clearance (Fig. 7), a statistically significant difference could not be demonstrated between control E_{PAH} and E_{PAH} at progressively increasing levels of sodium excretion in either group, and there was no significant difference between normal and caval dogs at any level (p value for all combinations was > 0.05 and often > 0.1). In addition, papillary tip osmolality in caval dogs was at least as great as in normal dogs at all levels of sodium excretion (Fig. 9; Table I), so that even if there was a greater tendency for increased medullary blood flow in caval dogs during saline diuresis, significant "wash-out" of medullary solute did not occur.

The lower peak $T^c_{H_2O}$ in caval dogs also cannot be explained by a defect

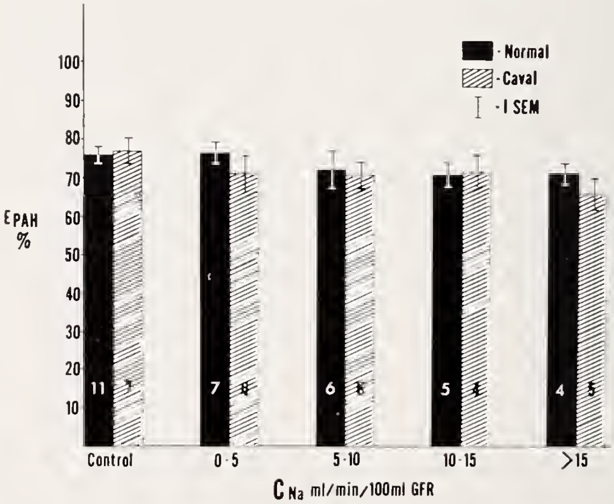

FIG. 7. The effects of 2% NaCl infusion on para-aminohippurate extraction (E_{PAH}). The data are grouped into successive 5 ml/minute increments of sodium clearance (C_{Na}). The shaded column represents the mean ± 1 SEM in normal dogs, and the hatched column represents the mean ± 1 SEM in caval dogs. The number in the column represents the number of experiments in each group.

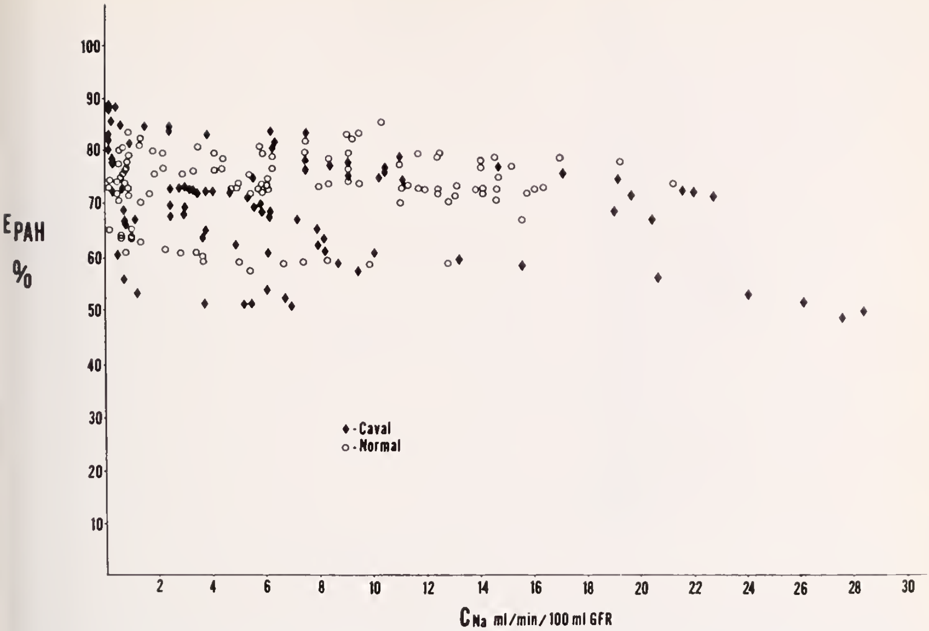

FIG. 8. The relationship between para-aminohippurate extraction (E_{PAH}) and sodium clearance (C_{Na}) during 2% NaCl infusion. The solid diamonds and open circles represent all the clearance periods obtained from seven experiments in caval dogs and 11 experiments in normal dogs, respectively.

in water permeability in the collecting duct or by a decreased accumulation of urea in the tubular fluid or papillary tip in these animals. Under non-diuretic conditions, maximum urine concentration and papillary tip or urine urea concentration were similar in normal and caval dogs, and there was osmotic equilibration between the urine and papillary tip (Table I). At various levels of sodium clearance during 2% saline diuresis, urea concentration was similar in normal and caval dogs in both the papillary tip and urine.

The report of Clapp and Robinson (20), demonstrating that the fluid in the distal tubule remains hypotonic in the dog with little change throughout its length even in the presence of ADH, may provide the basis for explaining the lower $T^c_{H_2O}$ curve in caval dogs. Although sodium transport in the medullary portion of the ascending limb is probably the most important variable affecting urine concentration, it determines the degree of dilution of the fluid in the early distal tubule as well. It has been previously suggested that during osmotic diuresis under hydropenic conditions the leveling off and decrease in $T^c_{H_2O}$ (particularly the formation of a dilute urine) indicates the failure of osmotic equilibration in the distal tubule (13, 21). Under these circumstances, $T^c_{H_2O}$ would be an underestimate of the amount of free water absorption in the collecting duct. In light of the evidence for enhanced sodium reabsorption in the ascending limb noted during water diuresis, it seems reasonable to propose that the tubular fluid in the early distal tubule

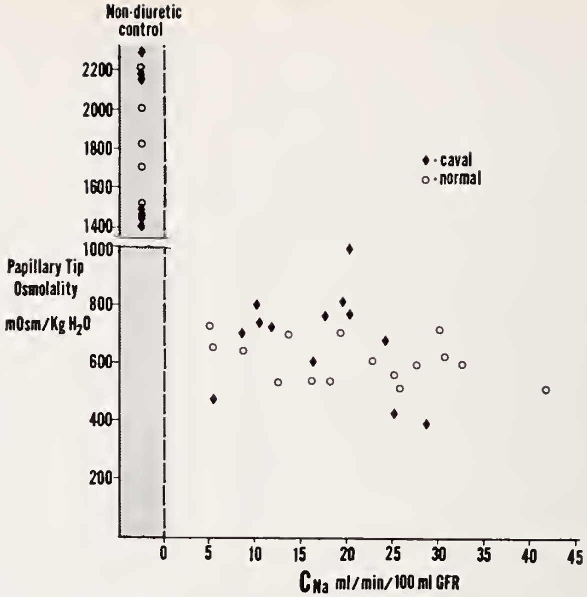

FIG. 9. Papillary tip osmolality under hydropenic non-diuretic conditions and at various levels of sodium clearance (C_{Na}) with 2% NaCl infusion. The osmolality represents "sum of osmoles," calculated by adding the urea concentration to twice the sum of the concentration of sodium, potassium, and ammonia, in mmoles/kg H_2O. The solid diamonds represent tissue obtained from caval dogs in six non-diuretic experiments and 13 diuretic experiments. The open circles represent tissue obtained from normal dogs in six non-diuretic experiments and 16 diuretic experiments.

TABLE I

Urine and Papillary Tip Osmolality—Non-Diuretic and during NaCl Diuresis†*

	Non-Diuretic	2% NaCl Diuresis	
		C_{Na} Less than 15 ml/min/100 ml GRF	C_{Na} More than 15 ml/min/100 ml GFR
Normal Dogs	(6)‡	(5)	(11)
Urine	1766 ± 134	552 ± 27	447 ± 22
Papillary tip	1789 ± 174	580 ± 32	581 ± 20
p value	N.S.**	N.S.	<0.001
Caval Dogs	(6)	(5)	(8)
Urine	1796 ± 82	493 ± 43	371 ± 19
Papillary tip	1837 ± 114	650 ± 36	666 ± 93
p value	N.S.	<0.02	<0.01

* calculated as "sum of osmoles," obtained by adding the concentration of urea to twice the sum of the concentrations of sodium, potassium, and ammonia in mmoles/kg H_2O.

† all data given as mean ± one standard error of the mean.

‡ represents the number of experiments in each group.

** not significant.

is more dilute in caval dogs than normal dogs. Under non-diuretic conditions, osmotic equilibration finally occurs (in the collecting duct) and maximum urine concentration is similar in both groups (Table I). During progressive osmotic diuresis, osmotic equilibration between urine and papillary tip does not take place in caval dogs at relatively low levels of sodium excretion (Table I) because of the increasing volume of a more dilute fluid reaching the collecting duct and, therefore, calculated $T^c_{H_2O}$ levels off and decreases earlier than in normal dogs. With increasing sodium excretion osmotic equilibration is no longer achieved in normal dogs (Table I), so that $T^c_{H_2O}$ eventually levels off or even shows a tendency to decrease in this group as well (Fig. 5). Although the comparison of papillary tip and simultaneous urine osmolality was carried out during saline diuresis only, the course of events is probably similar during mannitol diuresis. The higher $T^c_{H_2O}$ in caval dogs during mannitol diuresis compared to saline diuresis (and the same tendency in normal dogs) could be explained by the higher osmolality in the early distal tubular fluid at any level of sodium clearance because of the presence of a non-absorbable solute. In the rat, micropuncture experiments have demonstrated that the fluid in the early distal tubule is more dilute during saline diuresis than mannitol diuresis (22), indicating greater fractional sodium reabsorption in the ascending limb. In the rat, however, $T^c_{H_2O}$ is higher during saline diuresis because the tubular fluid becomes isotonic in the distal tubule prior to entering the collecting duct during both types of diuresis (22), in contrast to the finding in the dog (20).

It seems contrary to all previous concepts to propose that the magnitude of $T^c_{H_2O}$ is limited by increased sodium reabsorption in the ascending limb. However, it is quite likely that the classical concept is still applicable, namely that back-diffusion of water in the collecting duct is primarily dependent on ascending limb sodium reabsorption, since the actual amount of water back diffusing in the collecting duct may be greater in the caval dog, but only a part of it is calculated as $T^c_{H_2O}$. This would also explain the similar medullary osmolality and sodium concentration in caval and normal dogs (Figs. 9 and 10; Table I), despite the greater amount of sodium reabsorbed in the ascending limb in the former, inasmuch as there would be a greater "washout" of interstitial solute as more water back diffused from the collecting duct.

In conclusion, examination of the urinary diluting and concentrating operations in normal and caval dogs indicates that there is enhanced sodium reabsorption in the proximal tubule and ascending limb of Henle's loop in caval dogs. Furthermore, the data presented suggest that the apparent leveling off and decrease in $T^c_{H_2O}$ at higher levels of solute excretion during osmotic diuresis in the dog is due to progressive failure of equilibration between final urine and papillary tip osmolality, and that this phenomenon, in turn, is dependent in part upon the degree of hypotonicity present in the early distal tubule.

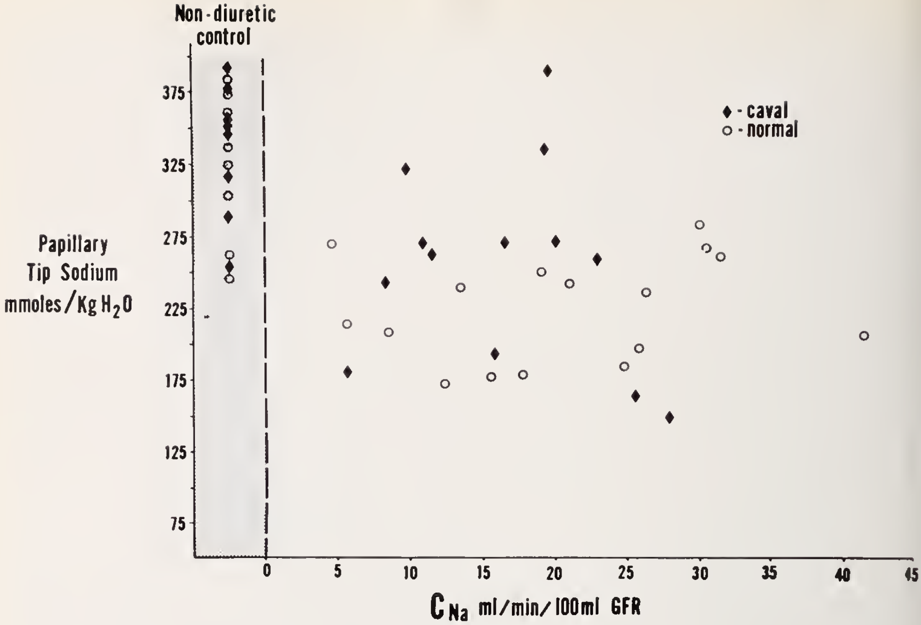

FIG. 10. Papillary tip sodium concentration under hydropenic non-diuretic conditions and at various levels of sodium clearance (C_{Na}) with 2% NaCl infusion. The solid diamonds represent tissue obtained from caval dogs in six non-diuretic experiments and 13 diuretic experiments. The open circles represent tissue obtained from normal dogs in six non-diuretic experiments and 16 diuretic experiments.

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The Regulation of Renal Sodium Transport by Intrarenal Physical Forces: A Resumé

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During the past few years, considerable progress has been made in understanding the mechanisms which regulate sodium and water reabsorption by the renal tubules. A surge of interest in this field was triggered by the observations of De Wardener and his co-workers (1, 2), who demonstrated that the natriuresis which follows a rapid intravenous saline infusion can occur in the absence of either a rise in glomerular filtration rate or a decrease in adrenocortical hormone activity. It was clear from their experiments that the kidney possesses another mechanism for changing the rate of sodium and water reabosrption by its tubules in response to acute changes in extracellular fluid volume, the so-called third factor. These observations were amply confirmed in many other laboratories (3–8), and it soon became evident that this newly discovered regulatory mechanism is of prime importance among the various factors influencing sodium excretion. For example, Levinsky and Lalone (4) found that urinary sodium and water excretion can increase in response to a saline load even when the filtered load of sodium is reduced by constriction of the renal artery, and sodium-retaining adrenocortical hormones are present in abundance. The importance of such a potent inhibitor of sodium reabsorption, which is evidently essential for the maintenance of body homeostasis, was thus made clear.

Although the precise nature of this regulatory mechanism is still not entirely understood, an increasing body of experimental evidence suggests that it may be related to the physical forces exerted by hydrostatic and colloid oncotic pressure gradients across the wall of the peritubular capillaries (9–16). Earley and Friedler (12) demonstrated that drug-induced dilatation of the renal blood vessels in non-diuretic dogs leads to an inhibition of sodium reabsorption, and that subsequent elevation of systemic blood pressure in these animals further inhibited sodium reabsorption. They postulated that these experimental maneuvers had resulted in an increased transmission of hydrostatic pressure across the glomerulus to the peritubular capillaries, and that the altered pressure gradient between the capillaries and the interstitium had slowed the removal of the tubular reabsorbate. In subsequent experiments, Martino and Earley (14) found that the response of the renal tubules to extracellular volume expansion correlated with the effect such expansion had on systemic blood pressure, renal vascular resistance, and the colloid oncotic pres-

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sure of the plasma. Isotonic saline infusion resulted in a natriuresis associated with dilution of plasma proteins and a rise in blood pressure. When albumin was administered to restore the colloid oncotic pressure toward normal, the diuresis was blunted considerably. Expansion of extracellular fluid volume by plasma infusion led to a natriuresis, but in this case the inhibition of sodium reabsorption was associated with increased blood pressure and a marked decrease in renal vascular resistance. Whole blood infusions did not lead to a natriuresis, and they attributed this to the fact that renal vascular resistance usually increased, whereas colloid oncotic pressure remained unchanged. These observations were consistent with the view that inhibition of sodium reabsorption depends upon either a decrease in colloid oncotic pressure, or an increase in hydrostatic pressure in the peritubular capillaries.

More direct information about the mechanism by which these physical forces determine sodium reabsorption was obtained by Lewy and Windhager (15), using micropuncture techniques. These authors produced a rise in peritubular capillary pressure by partially constricting the renal vein. The pressure rise was documented by direct measurements in the peritubular capillaries. Contrary to what might be expected, the proximal tubules were not compressed by the increase in surrounding pressure, but remained normally patent. Sodium transport rate, measured by both the split oil drop technique and tubular fluid/plasma inulin ratios (TF/P_{In}), was found to be markedly inhibited. Since this inhibition occurred in the absence of a proportional decrease in proximal tubular diameter, a mechanism other than that proposed by the tubular geometry hypothesis (17–19) seemed to be operating. This hypothesis contends that net sodium transport in the proximal tubule is determined by a function of the cross-sectional area of the tubule and is directly proportional to this area.

Koch, Aynedjian, and I (16) were also interested in studying the effect of peritubular physical forces with micropuncture techniques, but we used a different experimental protocol. We studied the effect of an acute elevation of systemic blood pressure, induced by bilateral carotid artery ligation, on proximal sodium transport in the rat. It had been demonstrated previously that acute increases in renal perfusion pressure produced mechanically in pump perfused kidneys inhibit sodium transport by the tubules (9–11). When the carotid arteries of the rat were ligated, sodium excretion and urine flow increased strikingly within a few minutes of the onset of hypertension. Hydrostatic pressure in the proximal tubular lumen was found to rise significantly from a mean control value of 16.2 cm H_2O to 28.1 cm H_2O, and we assumed that an equivalent rise in pressure had also occurred in the peritubular capillaries. Proximal sodium transport, measured by two independent micropuncture methods, was found to be markedly reduced. Again, as in the study by Lewy and Windhager (15), changes in tubular diameter could not account for the decrease in sodium reabsorption since the proximal luminal diameter actually increased from 18.5μ to 23.5μ at the same time that the sodium transport rate fell. It therefore seemed clear from these two micropuncture studies

(15, 16) that the tubular geometry hypothesis was not an adequate explanation for the inhibition of sodium transport which occurs with elevation of peritubular capillary hydrostatic pressure. In looking for alternative mechanisms, we investigated the possible role of aldosterone and the renin-angiotensin system (16). The intravenous administration of d-aldosterone did not prevent the inhibitory effect of acute hypertension on proximal sodium transport. Similarly, depletion of renal renin by chronic oral saline loading had no effect on the results. However, when the aorta was constricted above the renal arteries at the same time that the carotid arteries were ligated, in order to maintain renal perfusion pressure within a normal range, sodium reabsorption in the proximal tubule remained perfectly normal. This experimental maneuver accomplished two things: It excluded an extrarenal natriuretic hormone as the mechanism of sodium inhibition, and it provided further evidence that the rise in renal perfusion pressure was in itself the important initiating stimulus for sodium inhibition. Finally, in additional experiments, we found that removal of the renal capsule before hypertension was induced prevented the inhibition of proximal sodium transport in the superficial cortical nephrons (16). Since stripping the renal capsule alters the normal anatomical limits of the interstitial fluid surrounding the surface nephrons, this observation suggested that some change in the interstitial compartment of the kidney subsequent to the rise in peritubular capillary pressure is a prerequisite for the inhibition to occur.

These micropuncture experiments were thus in accord with the view of Earley and his co-workers (12–14) that peritubular physical forces can play a primary role in altering the rate of sodium transport by the proximal tubules. They further indicated that the effect of these forces could be very potent, since the magnitude of inhibition which was found in the proximal tubule was as great as 50 percent. In our (16) study, the rapidity of the change in sodium transport after acute hypertension was induced provided strong evidence that a hormone was not involved. Finally, it was clear from both our study and from that of Lewy and Windhager that significant exceptions exist to the proposed relation between sodium transport and tubular diameter. Indeed, these observations cast serious doubt upon the validity of the tubular geometry hypothesis itself.

The micropuncture studies just described demonstrated that the intrinsic reabsorptive capacity of the proximal tubular epithelium could be reduced by alterations of peritubular forces in normally hydrated, non-diuretic rats. Rector, et al (20) had found that saline loading also reduces the intrinsic reabsorptive capacity of the proximal epithelium, and that this reduction contributes to the natriuresis which occurs under these experimental conditions. However, it was not clear whether the effect on the proximal tubule was due to a natriuretic hormone released during extracellular volume expansion, to the same physical forces demonstrated to operate in hydropenic animals (15, 16), or to both. Many investigators have postulated that a natriuretic hormone is secreted in response to an increase in extracellular fluid volume, and some experimental evidence for such a hormone has been obtained in cross-circulation experiments in dogs (21, 22) and in micropuncture experiments in rats (23). In order to study this problem, Koch, Aynedjian, Aras, and I (24) carried out micropuncture experiments in saline loaded rats. We reasoned that if the rise in intrarenal pressure expected with saline loading was prevented, the comparative influence of physical force changes and a hormonal inhibitor could be evaluated. The protocol was as follows: Renal perfusion pressure was lowered to the range of 70–90 mm Hg by partial constriction of the aorta above the renal arteries. Ringer's solution was then infused intravenously at the rapid rate of 0.5 ml/minute for 50 minutes to expand ECF volume. Sodium reabsorptive rate was then measured in the proximal tubule, using the split oil drop technique, while renal perfusion pressure remained at the reduced level. During the second period, the aortic clamp was released, perfusion pressure allowed to rise, and proximal sodium reabsorption measurements repeated. Finally, in a third period, the aortic clamp was retightened to the same degree as in the first period, and split drop measurements again carried out. In addition to the split drop measurements, the velocity of tubular fluid flow in the unobstructed tubules of the kidney was also determined in the three experimental periods by measuring Lissamine green transit time. This measurement, together with the split drop measurements, allows one to calculate fractional reabsorption of sodium and water at the end of the proximal convoluted tubule (17, 19).

We found that during the first and third periods of the experiment, the rate of sodium transport by the proximal tubule was not significantly different from that in normal hydropenic animals. When the aortic clamp was released, an immediate and very marked inhibition of sodium transport was observed in the split drop measurements. Calculated fractional reabsorption at the end of the proximal tubule was normal during the two periods of reduced renal perfusion pressure and fell strikingly only when the clamp was released. Hydrostatic pressure in the proximal tubules was found to be approximately the same as in normal hydropenic animals during aortic constriction, but rose strikingly when the aortic clamp was released (24).

It seemed from these experimental observations that massive saline infusion had little or no effect on proximal sodium transport if the usual rise in intrarenal pressure that occurs with saline loading is prevented. Since a reduction in renal perfusion pressure should not have interfered with the action of a circulating natriuretic hormone, we concluded that the inhibition of the intrinsic reabsorptive capacity of the proximal tubule seen in saline loading is not due to a hormone, but is due rather to changes in peritubular physical forces. The rapidity of the change in proximal sodium transport when renal perfusion pressure was changed (within 2–3 minutes) also favored a physical rather than a humoral effect. However, in order to pursue this possibility further, we collected blood from saline loaded donor rats during and after release of aortic constriction. The collected plasma was dialyzed in the cold, and the dialysate injected into the proximal tubules of hydropenic assay rats for split drop measurements. No inhibition of sodium transport was observed with any of these plasma dialysates. Although these latter experiments offer additional suggestive evidence against the role of a humoral inhibitor of proximal sodium transport, they do not exclude the possibility of a non-dialyzable natriuretic hormone, or one that exerts its effect from the peritubular side of the epithelium rather than the luminal side, or a hormone that acts specifically on the distal tubule.

The theory of regulation of sodium transport by peritubular forces postulates that pressure gradients develop between the capillaries and renal interstitium (12–14). However, it is doubtful that renal interstitial pressure can be measured directly with present day techniques, and measurements in the proximal lumen and peritubular capillaries under several different experimental conditions have failed to demonstrate a pressure gradient between these two structures (15, 25, 26). In order to determine whether pressure gradients do develop in response to acute changes in systemic blood pressure, Aynedjian, Bansal, Goldman, and I (27) have recently measured hydrostatic pressures in the efferent arterioles on the surface of the kidney and in the proximal tubular lumen before and during infusion of epinephrine. The efferent arteriole was chosen because of the technical ease and relatively greater reliability of pressure measurements in these large vessels, as compared with the small peritubular capillaries, and because one can assume that a pressure change in the arteriole is transmitted to the capillaries. These experiments were carried out under two different conditions: either 2.5% saline infusion, or 2% mannitol infusion. In the saline infusion experiments, solute free water reabsorption ($T^c_{H_2O}$) was measured, and in the mannitol infusion experiments, free water clearance (C_{H_2O}) was measured. The two infusions had very different effects on renal vascular resistance, as determined by renal blood flow and GFR measurements. Hypertonic saline infusion reduced both afferent and efferent resistance, whereas hypotonic mannitol either caused no change or increased afferent and efferent resistance.

We found that during control periods, when mean systemic blood pressure was between 110–120 mm Hg, no consistent pressure gradient was present between the proximal tubular lumen and the efferent arteriole. With the onset of hypertension due to epinephrine (mean blood pressure rose 25–50 mm Hg), hydrostatic pressure rose in both the efferent arteriole and the tubular lumen, but there was transiently a greater rise in the blood vessels than in the tubular lumen. This was found to occur whether renal vascular resistance was low (saline infusion), or high (mannitol infusion). If hypotonic mannitol was infused at a higher rate, in order to achieve urine flow rates as high as occurred with hypertension, but hypertension was not induced, a reversal of the hydrostatic pressure gradient was observed (that is, the pressure in the proximal lumen became significantly higher than in the efferent arteriole). These two different pressure gradients within the kidney were associated with different effects on distal sodium transport, as measured by $T^c_{H_2O}$ and C_{H_2O}. When pressure was higher in the blood vessels than in the tubular lumen, a striking inhibition was seen in both $T^c_{H_2O}$ and C_{H_2O}. When pressure was higher in the tubular lumen, as in the experiments with high rates of mannitol infusion, C_{H_2O} continued to rise as the rate of delivery of sodium to the loop of Henle increased. We conclude, therefore, that an acute elevation of systemic blood pressure does cause a transient hydrostatic pressure gradient to develop within the kidney, with a higher pressure in the vascular compartment than in the tubular lumen, and that this pressure gradient is causally related to an inhibition of sodium transport. Although the demonstrated inhibition of sodium transport in these experiments was in the ascending limb of Henle's loop, inhibition probably also occurred in the proximal tubule (16). Presumably, once a pressure change has been transmitted across the glomerulus to the efferent arterioles, the numerous anastamoses between the peritubular capillaries allows the new pressure to spread throughout a large portion of the intrarenal vascular bed. The sharp reduction in $T^c_{H_2O}$ which was observed suggests that hydrostatic pressure had increased in the vasa recta, since these vessels supply the medullary portions of the ascending limb responsible for $T^c_{H_2O}$ formation.

The precise mechanism by which a pressure gradient between the peritubular capillaries and renal interstitium alters net sodium transport is presently unknown. Earley and his co-workers (12–14) have postulated that increases in pressure within the capillaries delay the removal of the tubular reabsorbate, thus causing an increase in the interstitial volume of the kidney. An increased interstitial volume might create a layer of high sodium concentration within the basal labyrinth adjacent to the transporting membrane of the tubular epithelium, which might then enhance the passive back-diffusion of sodium into the cells, or more directly interfere with the pump mechanism itself (15, 28). In the single cell organism, Valonia ventricosa, salt transport across the cell boundary is inhibited when intracellular hydrostatic pressure is raised (29), suggesting that the level of hydrostatic pressure may in itself influence transport rates independent of any changes in an intermediate fluid compartment. Whether a similar mechanism operates in the mammalian kidney is not known. Obviously, further investigation in this area is needed.

The recent observations on sodium regulating mechanisms in experimental animals naturally raise the question of the possible role of peritubular physical forces in human disease. For example, the sodium retention in patients with congestive heart failure or cirrhosis of the liver could conceivably be related to these forces, since in both conditions renal vascular resistance tends to be high (30, 31). In congestive heart failure, efferent arteriolar resistance is often disproportionately increased (30), and the resulting rise in filtration fraction would be expected to increase the concentration of proteins in the peritubular capillaries. Such an increase might enhance the movement of tubular reabsorbate into the capillaries, and thus set the stage for sodium retention and edema formation. In patients with essential hypertension, an abnormal increase in hydrostatic pressure in the peritubular capillaries might occur, and this might relate to their ability to excrete a sodium load more rapidly than normotensive subjects (32–34). The absence of sodium retention and edema in patients with primary aldosteronism might also be related to an abnormally high hydrostatic pressure in the intrarenal blood vessels, since the low levels of angiotensin in these patients could conceivably diminish renal vascular resistance and allow greater transmission of renal artery pressure to the peritubular capillaries. The hypertension in these patients might also contribute to this transmission. Although one could extend these speculations, it is clear from these few examples that many intriguing questions have been raised by the recent observations on peritubular physical forces in experimental animals. Future investigations into their role in human disease should prove to be both exciting and rewarding.

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Urea Reabsorption in the Nephron of Man and Dog*

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Urea is a principal end product of protein metabolism in mammals and constitutes the chief nitrogen substance in the urine. Because of the key role played by the kidney in the elimination of this substance, the mechanism of renal excretion of urea has long been of interest to the renal physiologist. Since the blood concentration and renal clearance of urea have been used to measure kidney excretory function and because of its unique role in the mechanism of urinary concentration (1, 2), the clinical nephrologist has also been concerned with factors dealing with the renal handling of urea. In man and dog, the renal excretion of urea is generally considered to consist of glomerular filtration and passive back-diffusion in the tubules (3). Recently, a number of factors have been shown to modify the tubular reabsorptive process. These include glomerular filtration rate (4, 5), dietary protein intake (6, 7), and urine flow rate (4, 8). In addition, it has been suggested from experiments performed primarily in species other than man and dog that active reabsorptive and secretory processes may be involved in the renal transport of urea (9–15).

In the past several years we have been investigating the influence of changes in urine flow on urea excretion in man and dog (16–18). Based on these experiments and the current insight gained from micropuncture studies (2, 19, 20), it appears possible to explain certain changes in urea excretion occurring during rapidly changing urine flow rates reported by earlier workers (4). Moreover, urea reabsorptive capacity of separate segments of the nephron may be estimated and fractional urea excretion used as a "tubular marker" in identifying tubular sites at which sodium and water reabsorption has been altered.

Three groups of experiments were performed in normal subjects. In the first series of studies, urine collections were initially made during maximal hydropenia, and were continued thereafter as hydrating solutions were administered for the succeeding 3 to 4 hours. During hydropenia, the fraction of filtered urea excreted ($C_{ur}/C_{in} \times 100$) averaged 40 percent and rose to 65 percent during sustained hydration (Fig. 1). A similar alteration in the percent of filtered urea excreted had been noted by other workers in experiments performed in both man and dog (4, 5). In the transition from hydropenia to hydration, a sharp increase in urea excretion and $C_{ur}/C_{in} \times 100$ occurred (Fig. 1). This early transient rise in urea excretion noted in these studies closely resembled the previously unexplained phenomenon of "urea exaltation" reported in the dog by other investigators (4), and may be explicable by the movement of urea sequestered in the renal medulla into the tubular fluid as urine flow rate

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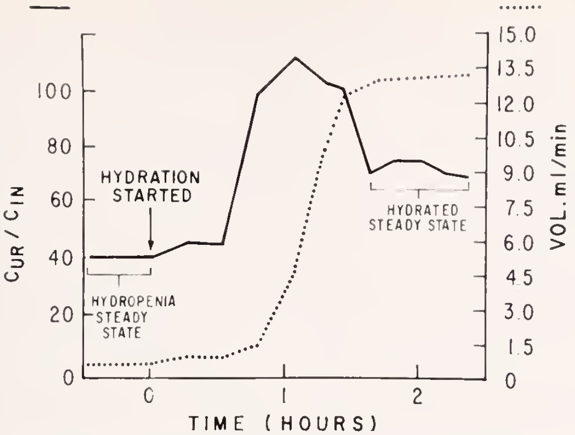

Fig. 1. Typical experiment showing changes in urea excretion (C_{ur}/C_{in}) and urine flow rate (V) produced by rapid hydration of a maximally hydropenic subject.

increases. It has been demonstrated in micropuncture studies that, at low urine flow rates (presence of antidiuretic hormone—ADH), the tubular fluid to plasma urea and tubular-fluid to plasma-inulin ratios in the late proximal tubule averaged 1.5 and 3.0, respectively, while these ratios in the first portion of the distal tubule were 7.7 and 6.9, respectively (20). These findings indicate that more urea leaves the medulla via the loop of Henle than enters from the proximal tubule. Analysis of late distal tubular fluid revealed the tubular-fluid to plasma-urea and tubular-fluid to plasma-inulin ratios to be 10.5 and 14.9, respectively (20). The finding that the urea ratio divided by the inulin ratio is approximately 0.7 in the late distal tubule (indicating that 70 per-cent of the filtered urea is present at this site) suggests that, even in the presence of ADH, urea movement from the distal tubule is limited. As the urea-rich, isosmotic fluid from the distal tubule enters the collecting duct, water reabsorption occurs in order to establish osmotic equilibrium with the surrounding hypertonic medullary interstitium (2). Under these circumstances, the urea concentration in the collecting duct fluid would rise, and urea would pass from the collecting duct into the medullary interstitium. The micropuncture data described above indicate that much of the medullary urea diffuses into the tubular fluid in the descending limb of the loop of Henle and is recirculated. This recirculation of urea through the medulla from the collecting duct to the loop does not change urea excretion under steady-state conditions, but does enhance the urea concentration within the distal tubular fluid. When hydration is rapidly produced, the back-diffusion of water from the distal tubule decreases, so that the urea concentration within the tubular fluid of the distal tubule falls. In addition, in the absence of ADH, the permeability of the collecting duct to urea apparently diminishes (21). Under these circumstances, urea movement from the collecting duct into the medulla would be minimal. This abrupt interruption of urea movement into the medulla would cause a rapid rise in urea clearance as urea continues to diffuse out of the medulla into the loop. The apparent increase in urea clearance ("urea exaltation"), would, by necessity, be a transient event, lasting only until the urea in the medullary interstitium was dissipated.

Since the medullary stores of urea would be diminished soon after high urine flows are produced, the sustained increment in urea excretion of 65 percent noted in the steady state of hydration (Fig. 1) cannot be attributed to a continued loss of urea from the medulla. There is some evidence to suggest that the increase in the percent of filtered urea excreted during hydration results from a fall in distal tubular urea reabsorption. Since hydration interferes with urea recirculation (vide supra) and increases tubular fluid volume, the distal tubule to plasma urea concentration gradient will diminish. In addition, hydration will increase tubular fluid flow rate and thereby decrease tubular-fluid to tubular-cell contact time in the distal tubule. These circumstances would tend to diminish the diffusion of urea from the distal tubule. Finally, the state of hydration, which decreases collecting duct permeability to urea (21), may similarly decrease distal tubular permeability to urea. In order to test the proposal that urea reabsorption in the distal tubule is curtailed during hydration, a second series of experiments were performed in which diuretic agents known to block sodium and water reabsorption at specific tubular sites were administered to subjects undergoing maximal water diuresis. If, during sustained hydration, urea reabsorption is confined to the proximal tubule, only those diuretic agents which block proximal tubular sodium and water reabsorption would be expected to alter the percent of filtered urea excreted.

When agents known to diminish proximal tubular solute and water reabsorption (mannitol, acetazolamide) (22, 23) were administered to hydrated subjects, a consistent rise in $C_{ur}/C_{in} \times 100$ was observed (Fig. 2). In contrast, the administration of compounds whose main site of action resides beyond the proximal tubule (organomercurials, ethacrynic acid) (24–27) failed to produce any rise in $C_{ur}/C_{in} \times 100$ (Fig. 2). Both the proximal and distal tubular agents caused comparable decrements in glomerular filtration rate. It seems unlikely, therefore, that the dissimilar effects noted on the percent of filtered urea excreted following the administration of these agents could be attributed to any alteration in renal hemodynamics. These studies thus support the proposal that in the state of sustained hydration, urea reabsorption is confined to the proximal tubule.

A third group of experiments was performed in order to determine whether, in the presence of ADH, urea reabsorption in the distal tubule is limited during periods of high urine flow rate. In these studies, a 10% mannitol infusion containing vasopressin was administered to subjects until urine flow had stabilized at a level comparable to that achieved in the hydrated state described above. After 3 to 4 control urine collections had been obtained, agents acting in the proximal tubule (mannitol, acetazolamide) or distal tubule (mercaptomerin) were administered (Fig. 3). The pattern of urea excretion in these studies was found to be identical to that noted in the hydrated experi-

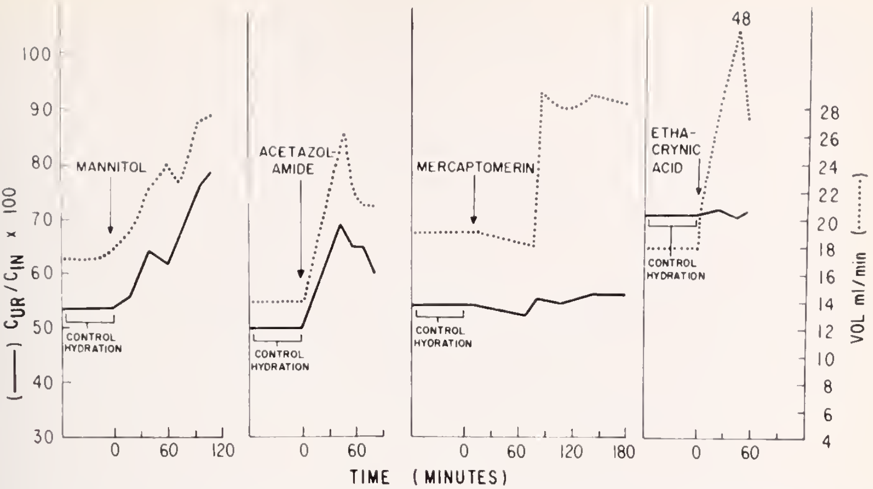

Fig. 2. Four typical experiments depicting the changes in urea excretion (C_{ur}/C_{in}) and urine flow rate (V) associated with a diuresis produced by a 3% mannitol solution, acetazolamide, mercaptomerin, and ethacrynic acid administered to hydrated subjects after urea exaltation had subsided.

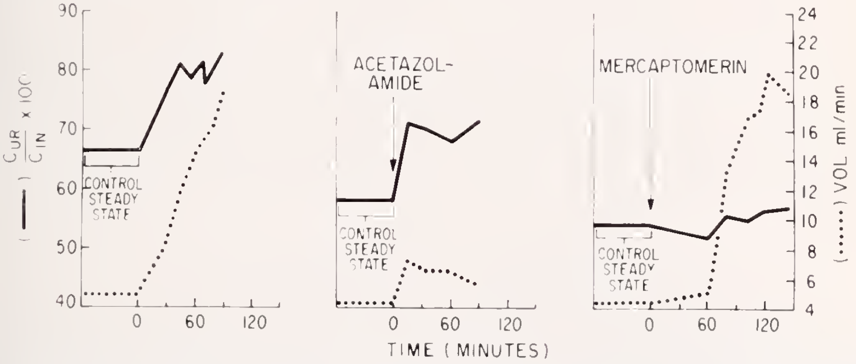

Fig. 3. Three typical experiments showing changes in urea excretion (C_{ur}/C_{in}) and urine flow rate (V) produced by the rapid infusion of a 10% mannitol solution, acetazolamide, and mercaptomerin in three hydropenic subjects during the course of a steady modest mannitol diuresis.

ments. Specifically, the proximal tubular diuretics produced a consistent increase in $C_{ur}/C_{in} \times 100$, while the distal tubular agents did not alter this parameter (Fig. 3). These experiments indicate that, despite the presence of ADH, little urea is reabsorbed in the distal tubule at high urine flow rates. It is proposed that high urine flow rate limits back-diffusion of urea in the distal tubule by diminishing tubular-fluid to tubular-cell contact time and by decreasing the urea concentration within the distal tubule.

It would appear then that during high rates of urine flow, urea reabsorption virtually ceases from the distal tubule and is apparently confined to the proximal tubule. Approximately 35 percent of the filtered urea is reabsorbed during periods of high urine flow, while 60 percent is reabsorbed during hydropenia

(Fig. 1). The 25 percent increment in urea reabsorption under conditions of low urine flow must represent the maximum amount of urea reabsorbed in the distal tubule during hydropenia. The findings that a modest fraction of filtered urea is reabsorbed in the distal tubule during hydropenia and little or none is reabsorbed in this segment under conditions of high urine flow suggest that, in man, the distal tubule, in comparison to the proximal tubule, is relatively impermeable to urea. This proposal agrees with conclusions of other investigators from studies performed in man (3), rat (20), and dog (28, 29).

Stop-flow experiments performed in the dog also support the proposal that the distal portion of the nephron is quite impermeable to urea during periods of high urine flow, even in the presence of ADH. After 3 minutes of ureteral obstruction in animals receiving saline infusions with added vasopressin, it was noted that only a small increase in the fraction of the available sodium and water was reabsorbed in specimens obtained from the most distal nephron (Fig. 4). Coincidentally, there was little loss of urea in these specimens. Sodium and water reabsorption was increased in more proximal specimens, however, and was accompanied by enhanced urea reabsorption. When sodium and water reabsorption was blocked throughout the distal nephron by the administration of ethacrynic acid, urea reabsorption virtually ceased (Fig. 4). These findings indicate that increased reabsorption of available urea does not occur in the distal nephron despite the prolonged tubular-fluid to tubular-cell contact time unless a favorable transtubular urea concentration gradient is established by the reabsorption of sodium and water. The stop-flow experiments in dog therefore support the conclusion suggested by the clearance studies in man that the distal nephron (unlike the collecting duct) is quite impermeable to urea.

Conclusions and Summary

The present studies suggest that the reabsorption of urea throughout the nephron of man and dog is passive and dependent on the reabsorption of sodium and water, that the distal nephron's urea reabsorptive capacity is quite limited, and that the major fraction of urea reabsorption takes place in the proximal tubule. These conclusions would explain the conspicuous increase in blood urea concentrations often noted in those disease states associated with edema formation (congestive heart failure, decompensated cirrhosis, and nephrosis). It has been suggested that in these states enhanced fractional reabsorption of the filtered load of sodium and water occurs within the proximal tubule (30, 31).

Methods available for localizing the specific tubular sites at which sodium and water reabsorption is altered in man are limited to analysis of changes in solute free water excretion (C_{H_2O}) and solute free water reabsorption ($T^c_{H_2O}$) (24–26). The marked difference in urea reabsorption within the proximal and distal tubule reported in the present studies indicates that analysis of changes in fractional urea excretion might prove useful as a "tubular marker." Specifically, during periods of sustained high urine flow, changes in sodium and

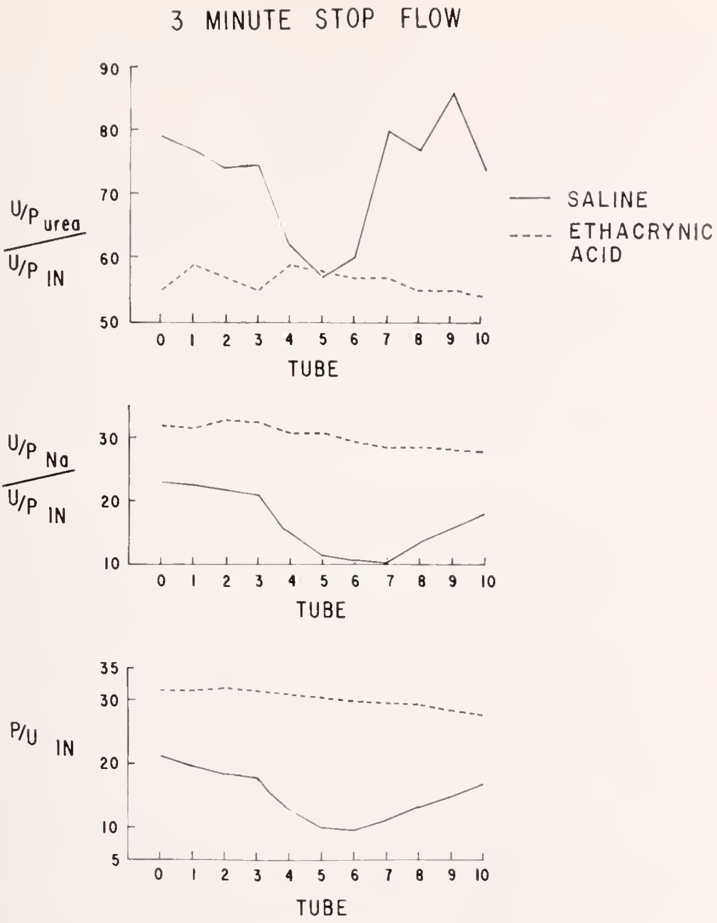

Fig. 4. Typical experiment showing the changes in the fraction of filtered urea ($U/P_{urea}/U/P_{In}$), sodium ($U/P_{Na}/U/P_{In}$), and water (P/U_{In}) after release of 3 minutes of ureteral obstruction during the course of saline infusion, with and without the administration of ethacrynic acid. On the abscissa is plotted the free-flow values (0) and the successively collected specimens.

water reabsorption in the proximal tubule would be associated with parallel changes in urea reabsorption, while alterations in distal tubular sodium and water reabsorption would occur without any change in this parameter. It seems reasonable, therefore, to ascertain the tubular site(s) at which diverse physiologic and pharmacologic stimuli affect sodium and water transport in man by comparing changes in glomerular filtration rate, urea and sodium clearance, and water excretion during periods of high urine flow.

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Adult-Type Gaucher's Disease: A Secondary Disorder of Iron Metabolism*

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Gaucher's disease is a primary disorder of lipid metabolism characterized by an enzyme deficiency resulting in accumulation of glucocerebrosides (1, 2) within reticuloendothelial cells. In adults, the lipid is believed to originate from phagocytized erythrocytes (1–3). In such cases, some (4), or most of the abnormal cells stain with Prussian blue to varying degrees (5–8). By contrast, the cells of infants with the disorder are not stained by that procedure (9). Electron microscopic studies of Gaucher cells of adults have revealed the presence of ferritin even in individuals having no evidence of hemolysis (7, 8), suggesting that the normal destruction of red cells over decades, or ropheocytosis of iron from the extracellular fluid compartment may be sufficient to result in intracellular iron in the pathognomonic cells.

As some characteristic cells, despite having abundant lipid, do not stain with Prussian blue, the presence of intracellular iron cannot be a primary abnormality in this disorder. However, the existence of abundant iron demonstrable not only histochemically (4, 5, 7) but by electron microscopic (7, 8) and immunochemical (7) procedures, as well as by chemical analysis (10) accompanied by abnormal ferrokinetic studies (6, 8), indicates that Gaucher's disease in adults should be considered to be a secondary disorder of iron metabolism.

Materials and Methods

One fresh and six frozen surgically removed spleens from patients with the adult-type of Gaucher's disease and one frozen spleen from an infant with the disorder were studied. In addition, one bone marrow aspirate from a previously splenectomized nonanemic adult case was freshly obtained. Smears of all tissues were stained by May-Grunwald stain followed by Giemsa or the Prussian blue procedure (11). Spleen sections were stained by hematoxylin and eosin or by Prussian blue (12).

Freshly obtained bone marrow (one case) and spleen (one case) were fixed in cold buffered osmium tetroxide and embedded in Epon for electron microscopy using an RCA EMU 2B instrument.

Standard hematologic procedures were employed for cell counts.

The distribution of radioiron in a splenectomized nonanemic woman was followed following the intravenous injection of six μc of ^{59}ferrous citrate (sp.

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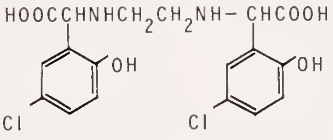
act. 9.5 mc/mg). The plasma disappearance and subsequent red cell uptake were studied using a well-type scintillation counter (Baird-Atomic). Localization of iron by surface counting over the liver, sacrum, and lower extremities was performed using a gamma-sensitive probe (Baird-Atomic) connected to a recorder (Texas Instruments). Almost 4 weeks later, an erythrocyte survival study was performed using 50 μc of $Na_2{}^{51}CrO_4$. Differential scintillation counting permitted recognition of the counts from each isotope present in the blood samples. All radioiron values were corrected for isotope decay. The level of radioactivity in blood samples was corrected for the minor variations in hematocrit value of each sample. Serum iron and iron binding capacity determinations were performed according to the methods of Ramsay (13) and Rath and Finch (14).

In vitro demonstration of the movement of iron into and out of Gaucher cells was performed using freshly obtained bone marrow from the same splenectomized nonanemic adult with Gaucher's disease. Marrow particles were suspended in Eagle's minimum essential medium (15) with added pyruvate, glutamate, and nonessential amino acids (Microbiological Associates) with or without transferrin, ferric chloride, or ferrous sulfate as sources of iron in physiologic concentrations (16, 17). The mixtures were incubated at 37°C for 4 and 25 hours. Removal of ferritin from Gaucher cells by chelating agents was studied by incubating marrow particles suspended in the fortified Eagle's medium with the sodium salts of three chelates at room temperature for up to 25 hours. These were diethylenetriaminepentaacetic acid (DTPA), Chel 138, and RA 322 (Geigy) (Fig. 1). The final concentration of each chelate was 5×10^{-5}M.

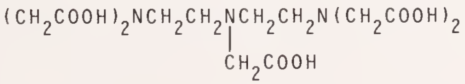
Results

Prussian blue staining of seven spleens and one bone marrow aspirate from adult cases of Gaucher's disease demonstrated iron within the characteristic



RA 322

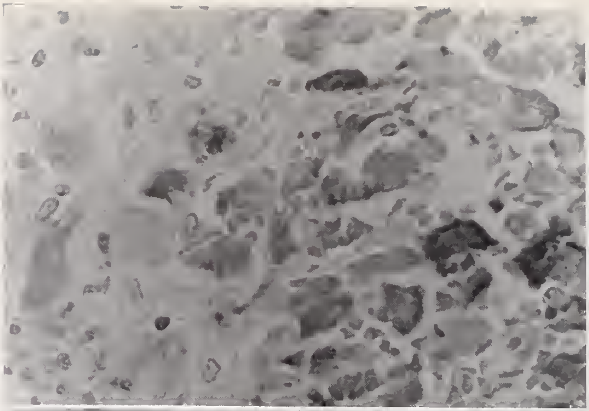
Chel 138 - same as RA 322 but no Cl groups



$$(CH_2COOH)_2NCH_2CH_2\underset{\displaystyle CH_2COOH}{\underset{|}{N}}CH_2CH_2N(CH_2COOH)_2$$

DTPA

FIG. 1. Formulae of three chelates used to investigate removal of iron from Gaucher cells *in vitro*.



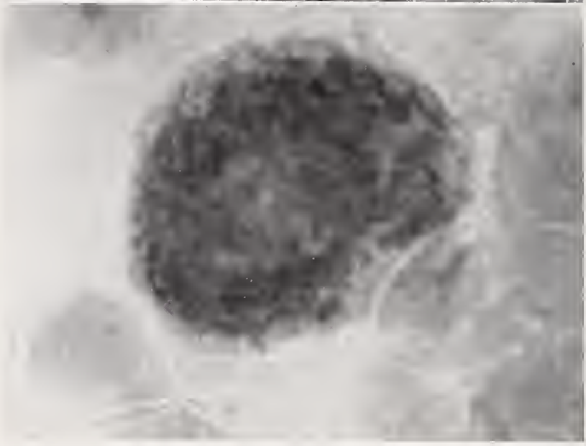


FIG. 2. Spleen section showing various quantities of iron in the numerous Gaucher cells. Some apparently have no stainable iron. Note the paucity of extracellular hemosiderin. Obtained from a male, age 46, with a platelet count of 23,000/mm^3. Hematocrit 45.5%, hemoglobin 15.0 gm%, reticulocytes 1.7%, serum iron 72 μg%, unsaturated iron-binding capacity 200 μg%, and white blood count 4,800/mm^3 (Prussian blue × 400).

FIG. 3. Bone marrow smear from the case whose data are tabulated in Table I. A Gaucher cell with appreciable iron, presumably ferritin because of its diffuseness (Prussian blue × 1,000).

cells in all. Examination of the spleen of a 1-year-old infant with the disorder revealed no iron. In the former, despite abundant intracellular iron, the extracellular metal was often sparse (Figs. 2 and 3).

Not all typical cells stain with Prussian blue (Fig. 2), so iron accumulation is not a basic defect in the lipid-containing cells. However, most Gaucher cells do contain ferritin which is visualized by electron microscopy (Fig. 4). In these cells, ferritin is diffusely distributed in the matrix and is not particularly associated with the zones rich in endoplasmic reticulum where the abnormal lipid presumably is concentrated. Ferritin is often prominent in the periphery of the cell, suggesting that it was either obtained from, or will be discharged into the interstitial fluid or plasma. Therefore, it was deemed advisable to study the fate of intravenously administered radioiron *in vivo* (Table I) and the

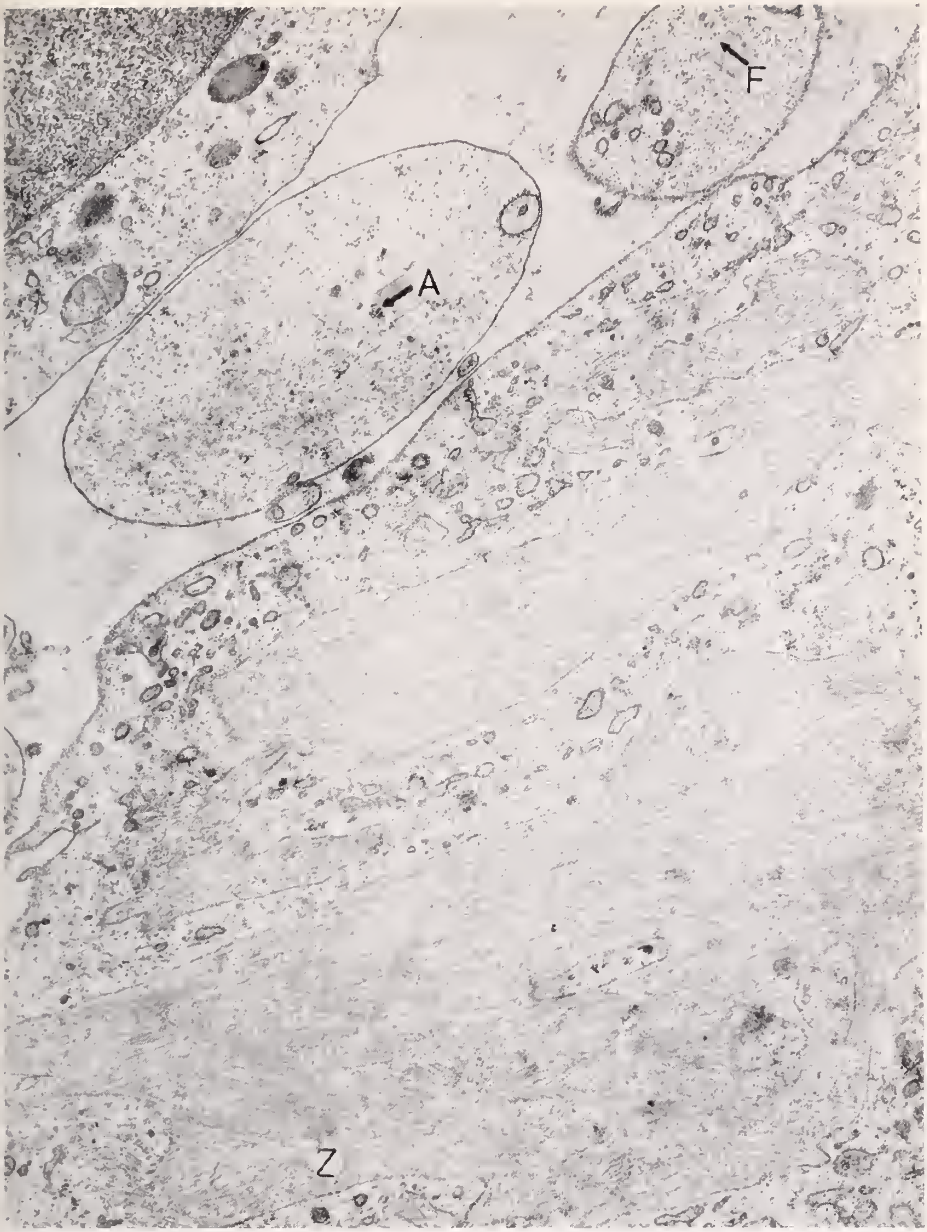


Fig. 4. Electron micrograph from the same spleen as in Figure 2. There are numerous individual ferritin micelles (F) and a few small ferritin aggregates (A). There are large cytoplasmic zones (Z) containing endoplasmic reticulum. The lipid is presumably located in those zones (× 30,000).

possible exchange of iron from the Gaucher cell with the surrounding medium *in vitro* (Tables II and III).

Fortunately, we were able to study in detail an adult with Gaucher's disease and an apparently normal erythron (Table I). Despite normal bone marrow

TABLE I

Hematologic and Ferrokinetic Data Obtained from a Nonanemic Case of Adult-Type Gaucher's Disease

(Female, Age 40, Weight 59.1 kg. Splenectomy for Thrombocytopenia Age 17; Hepatomegaly 4 cm. Pain, Radiolucencies, and Joint Disintegration in Right Hip and Knee)

Hematocrit	41.5%	White blood count	11,050/mm^3
Hemoglobin	14.0 gm%	Neutrophiles	49%
Reticulocytes	1.3%	Bands	10%
Platelets	204,000/mm^3	Metamyelocytes	3%
Sedimentation rate	7 mm/hour	Lymphocytes	27%
Bone marrow: normal except for moderate numbers of Gaucher cells		Monocytes	9%
		Eosinophiles	2%

	Patient	Normal	Reference
Serum iron (μg%)	86	70–180	17
Unsaturated iron-binding capacity (μg%)	179	120–290	17
Total iron-binding capacity (μg%)	265	300–360	17
^{59}Fe plasma disappearance T½ (minutes)	64	80–120	18
Fraction of iron removed from plasma per hour (%)	64.9	41.0	19
Plasma iron transport rate (mg/day/100 ml blood)	0.80	0.61	18
Red cell incorporation of ^{59}Fe (%)	59.0	80% or more	18
Plateau of red cell incorporation of ^{59}Fe (days)	4–5	5	17
Iron utilization for effective red cell production (mg/day)	19.4	23	18
^{51}Cr red cell survival T½ (days)	29.2	26–29	17
Red cell mass (ml/kg)	27.8	27.0	20
Plasma volume (ml/kg)	41.7	37.4	20

TABLE II

Movement of Iron into and out of Gaucher Cells in vitro

	Diffuse Staining of Gaucher Cells (Prussian Blue)	
	4 Hours*	25 Hours*
Bone marrow, minimum essential medium (MEM)	++	Trace
Bone marrow, transferrin, MEM	Trace	0
Bone marrow, FeCl$_3$, MEM	++	+
Bone marrow, transferrin, FeCl$_3$, MEM	+++	++
Bone marrow, FeSO$_4$, MEM	++	+++
Bone marrow, transferrin, FeSO$_4$, MEM	+++	+++

Bone marrow 0.15 ml. Minimum essential medium (Eagle) to a final volume of 1.1 ml. Transferrin (2.7 μg/ml) 0.25 ml. FeCl$_3$ or FeSO$_4$ (1 μg/ml) 0.25 ml.

* rotated during 37°C incubation. At 0 hours +++ diffuse staining of Gaucher cells.

TABLE III

Removal of Ferritin from Gaucher Cells by Chelating Agents in vitro

	Diffuse Staining of Gaucher Cells (Prussian Blue)	
	4 Hours*	25 Hours*
Bone marrow, minimum essential medium (MEM)	++	+
Bone marrow, DTPA, MEM	+	Trace
Bone marrow, Chel 138, MEM	0	0
Bone marrow, RA 322, MEM	+	Heavy trace

Bone marrow 0.15 ml. Minimum essential medium (Eagle) to a final volume of 2.0 ml. Initial chelate concentration 0.001 M. 0.1 ml added. Final chelate concentration 5×10^{-5} M.

* 25°C incubation. At 0 hours +++ diffuse staining of Gaucher cells.

cellularity, except for the characteristic cells, and normal hematocrit, hemoglobin, and reticulocyte values, the patient had a low normal serum iron level, a rapid plasma radioiron disappearance, and diminished incorporation of radioiron into her erythrocytes. This reached a plateau about 4 days after the intravenous administration of ferrous citrate. These ferrokinetic abnormalities occurred in the presence of normal erythrocyte survival and abundant intracellular iron.

Surface counting was performed on the day of isotope administration and frequently thereafter for 13 days, and again on the 23rd day after the isotope was administered (Fig. 5). There was a fall in counts over the sacrum until the red cell uptake reached its plateau. Counts over the liver tended to parallel those over the sacrum and were from 50 to 120 counts higher, except on the 6th day, suggesting a transfer of radioiron from the liver to the marrow. Thereafter, the liver counts were essentially unchanged until the 13th day. During this period the sacral counts fell. Despite radiolucencies in the right hip (Fig. 6), counts over the upper third of both femurs were similar. On the 23rd day, the counts over the femurs and the sacrum rose while the liver counts fell. This suggests a further net redistribution of the radioiron from the liver to the bone (Fig. 5). This was followed by a rise in red cell uptake of 5 percent above the initial plateau of isotope incorporation.

On the 13th day after radioiron administration, the probe was placed close to both greater trochanters. The counts over the right one were 196, and over the left were 177. On the 23rd day, the right lower femur had 228 counts, and the corresponding position on the left had 203 counts. This suggests that slightly more radioactivity was present over the right hip (Fig. 6) and knee regions, where presumably more Gaucher cells were present.

To determine by *in vitro* methods whether iron may enter and leave the Gaucher cells, bone marrow from the patient who was studied *in vivo* with radioiron was placed in an iron-free culture medium (Table II). It was observed that iron progressively left the Gaucher cell after 4 and 25 hours incubation. This egress was hastened by having transferrin in the medium. Even in

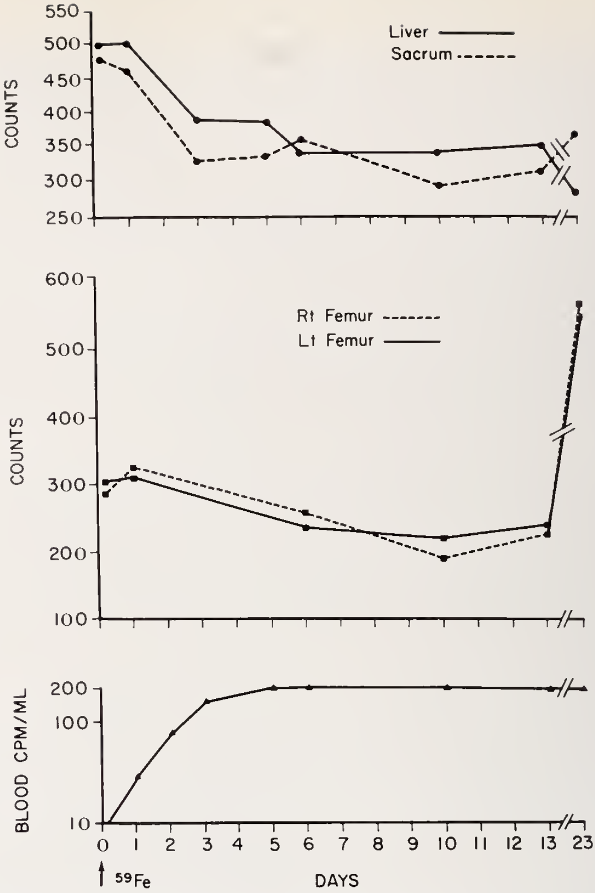


FIG. 5. Surface counting data obtained over liver and sacrum (top) and the upper thirds of both femurs (middle). For comparison, counts of blood samples obtained using well-type scintillation equipment are at the bottom. Other laboratory values of this patient are in Table I.

the presence of trivalent inorganic iron at physiologic iron concentrations, ferritin left the cells. However, the net turnover was negligible after 4 hours' incubation when transferrin was added. On prolonged incubation in the presence of $FeSO_4$, the cells contained more iron as judged by the diffuse blue color after Prussian blue staining. This contrasted with what was observed in tubes containing bone marrow alone or with transferrin, as well as in those having $FeCl_3$.

Addition of chelates (Table III) decreased the observed Prussian blue staining intensity after 4 and 25 hours compared to the control tube containing just marrow and medium. Incubation alone decreased the staining intensity of the marrow compared to the fresh specimen prior to incubation. Therefore, in an iron-free medium ferritin is able to leave the Gaucher cells. This

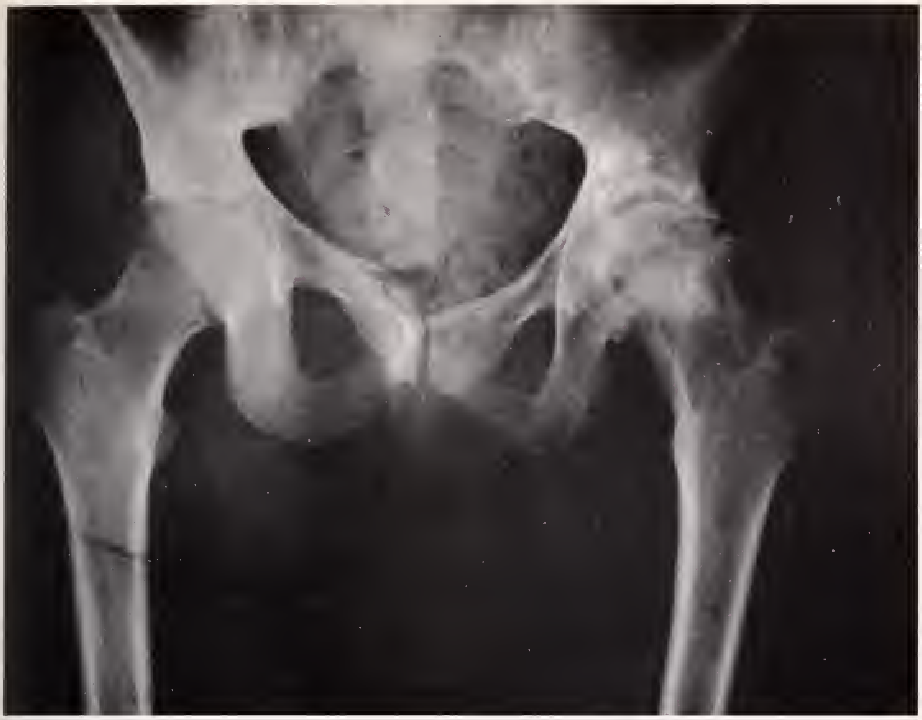


Fig. 6. X-ray of pelvis showing marked degenerative changes with bone rarefaction and deposition in the hip joint region and lytic areas in the femur. The patient's right side is affected (Table I).

process is hastened by various chelating agents (Table III) as well as by transferrin (Table II).

Discussion

Large amounts of ferritin give a bluish hue to a cell stained by the Prussian blue procedure (5, 21, 22). This color also occurs when ferritin is stained *in vitro* (5, 23). Recommendations (24, 25) that tissue iron be estimated from unstained smears should be inapplicable to Gaucher's disease, as the diffuse intracellular ferritin would not be observed and any lipofuscin pigment might be interpreted as hemosiderin in such smears.

The iron dispersed within the cells as individual micelles is ferritin. The large aggregates which comprise water-insoluble particles are usually termed hemosiderin (26). In Gaucher cells, such aggregates are rare. This general cytoplasmic dispersion of ferritin molecules has also been noted in normal macrophages (27), in reticuloendothelial cells of rabbits following intravenous saccharated iron oxide (28), and in hemochromatosis (29). Gaucher's disease has been compared to hemosiderosis because of the abundant reticuloendothelial cell iron in both conditions (30). Electron micrographs of Gaucher cells

(7) demonstrate an appreciable number of ferritin micelles in the periphery of the cytoplasm. This, together with the rapid disappearance of plasma radioiron, might indicate that they were derived not only from ingested red blood cells (7, 8, 31, 32), but from the surrounding plasma or interstitial fluid which normally contains a significant labile iron pool (33, 34).

Despite the abundance of intracellular iron, extracellular hemosiderin granules were often subnormal (Fig. 2), suggesting preferential localization of storage iron within the characteristic cells.

The rapid plasma clearance and reduced erythroid uptake of radioiron in this patient with normal peripheral erythroid values is similar to data obtained in hemolytic anemia (35, 36), and explained on the isotope having entered a large, slowly-exchanging iron pool within the reticuloendothelial system (35). The reduced iron utilization found in hemochromatosis (37) has also been explained on the basis of dilution in a greatly increased iron pool (38, 39). Rapid plasma clearance and poor red cell uptake was also described in a patient with Gaucher's disease by Loeffler (40). However, no data were given as to the presence or absence of anemia in his case whose serum iron level was not diminished. In contrast to Gaucher's disease, in hemolytic anemia the serum iron level is often elevated (36), and in hemochromatosis it is usually elevated (16).

A dynamic equilibrium has been described between plasma iron and ferritin iron (41). In normals, much of the plasma iron enters the reticuloendothelial system and the hepatic parenchyma (42–45). In Gaucher's disease, this entry into depots is believed also to involve the pathognomonic cells with their abundant iron content. This ability to exchange iron with the medium has been demonstrated *in vitro* (Tables II and III). If this likewise occurs *in vivo*, the corollary would be that not all the iron in those cells derives from erythrophagocytosis. If it did, the clearance of radioiron from the plasma would be normal.

Najean (46) has stated that the saturation of the prehematin iron compartment (easily exchangeable ferritin) located in erythroblasts and in the reticuloendothelial cells controls plasma iron clearance. This observation is particularly pertinent in Gaucher's disease, in which the number of reticuloendothelial derivatives is greatly increased. However, as a result of expansion of this compartment, in the absence of hemolysis its overall iron saturation would be reduced. Therefore, iron clearance from the plasma would be unusually rapid, as has presently been noted. This would secondarily reduce the quantity of radioiron available to the marrow for heme synthesis (47), thus diminishing the uptake of radioiron.

Once the radioiron enters the depots, its return is slow (48, 49), being appreciable only after 14 days (38). This may explain the reduced surface counts over the liver around 23 days after administration of isotope, with redistribution to the sacral marrow and apparently to areas of bone pathology in the right hip (Fig. 6) and knee regions. As this was unaccompanied by any elevation of the level of red cell uptake of isotope at that time, and only a 5 percent rise subsequently, it suggests that essentially all the isotope had entered a non-erythroid pool (Fig. 5).

The combination of diversion prior to reaching the erythron, dilution into an abnormally large and avid storage compartment, and retention by the latter is believed to explain the ferrokinetic data in adult variety Gaucher's disease.

Saltman, Fiskin, and Bellinger (50) observed that rat liver slices in an iron-free environment lost iron, as do Gaucher cells (Tables II and III). For iron to enter depot cells it must be divalent (51). After 25 hours' incubation with $FeSO_4$, iron-staining of Gaucher cells was more marked than when $FeCl_3$ was in the medium. However, the presence of transferrin prevented the net loss of iron from the cells after 4 hours' incubation with either inorganic salt (Table II). Ross, Kochwa, and Wasserman (52) have stated that both divalent and trivalent iron can combine with transferrin. In the absence of such salts, the iron-binding protein (Table II) and chelates (Table III) hasten the loss of the element from the pathognomonic cells. These observations suggest that transferrin, which is located both intra- and extra-vascularly (53), may be necessary for the exchange of iron between Gaucher cells and their environment. This would resemble iron exchange in rat liver slices (54), reticulocytes (55), erythroblasts, reticuloendothelial cells, and liver cells (41), and the placenta (56). In the congenital absence of transferrin, severe siderosis occurs (57). Therefore, iron can enter tissues even without that protein. However, this does not deny its usual importance in iron metabolism.

Lee, Balcerzak, and Westerman (8) performed ferrokinetic studies in Gaucher's disease. None of their four patients' erythrons were as hematologically normal as the present one, two having hematocrit values of 37 and 38 percent accompanied by increased iron absorption, while the other two had decreased red cell survival values. Nonetheless, our data are similar in that increased iron stores together with the low or low normal plasma iron level, rapid plasma disappearance, and low red cell uptake existed in 3 of their 4 cases as well as at present. They interpreted their data as "moderately increased ineffective erythropoiesis" because of the failure of surface counting to show initial accumulation of radioiron in organs containing a large number of Gaucher cells, despite the value of red cells produced per 100 ml of whole blood per day being normal in all but one of their patients. We prefer to interpret the findings on the basis of diversion of much of the radioiron to the storage cells, at first predominantly located in the liver, and later to those in the bone marrow, as evidenced by the present surface counting data, particularly of the greater trochanter and lower femoral regions in which marked degenerative changes occurred (Fig. 6). The present *in vitro* studies indicate that marrow incubated with physiologic amounts of transferrin and of iron stains more intensely with Prussian blue than when no iron is present (Table II). These observations suggest that Gaucher cells are able to take up the metal from their environment.

There is no need to postulate ineffective erythropoiesis in the present study. If much of the plasma radioiron entered the enlarged reticuloendothelial compartment, less was available for erythropoiesis. The total quantity of iron employed for hemoglobin synthesis, however, must have been normal, as evidenced by the patient's normal red cell mass, hemoglobin, and reticulocyte levels. Therefore, nonradioactive depot iron made up for the subnormal quantity of isotope which reached the erythron. Thus, in this disorder radioiron uptake does not truly reflect erythropoietic activity because, it is believed, much of the isotope was diverted into the Gaucher cell mass. Although erythroid marrow activity largely determines plasma iron turnover (58), in Gaucher's disease the avidity of the pathognomonic cells of reticuloendothelial origin must be recognized. Circulating hemoglobin level and marrow hemosiderin are stated to be the best criteria for total body iron (59). In Gaucher's disease the ferritin in the enlarged reticuloendothelial compartment must also be considered in appraising total body iron.

Abnormalities in serum acid phosphatase (60, 61), liver esterase (62), and serum proteins (63) have been reported in this disorder, although the basic defect is the inability of the reticuloendothelial cell to rid itself of the lipoprotein stroma of ingested blood cells (1, 2). It is believed that a sufficiently prominent abnormality in the distribution of iron likewise occurs to warrant considering the adult variety of Gaucher's disease to be a secondary disorder of iron metabolism.

Summary

Ferritin has been demonstrated in many, but not all, of the pathognomonic cells in tissues obtained from eight adults with Gaucher's disease. One adult, despite the absence of anemia or reticulocyte abnormalities, had a rapid plasma radioiron disappearance, with subnormal uptake into her red cells. *In vitro,* transferrin-mediated exchange of iron into and from Gaucher cells has been demonstrated. If this occurs *in vivo,* as suggested by surface counting, the ferrokinetic data may be explained by diversion of radioiron from the plasma to an avid, retentive storage depot within the numerous Gaucher cells, thereby depriving the erythron of much of the administered quantity of radioiron. Thus, abnormalities in ferrokinetics may occur which do not necessarily reflect erythropoietic activity, because the Gaucher cells themselves constitute a significant depot of storage iron.

Adult-type Gaucher's disease is a secondary disorder of iron metabolism, since in infancy the characteristic cells do not contain abundant non-heme iron. Therefore, it is not a primary defect in this disorder of lipid metabolism.

Acknowledgments

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Familial Polycythemia*

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Abnormal Hemoglobins and Familial Polycythemia

Familial polycythemia is a benign disorder, or group of disorders, in which erythrocytosis has occurred in more than one member of a kindred (1–3). White blood and platelet counts are normal, suggesting a difference from polycythemia vera, and the usual causes of secondary polycythemia have been eliminated as etiologic factors. Some patients have had *only* erythrocytosis, with normal hemoglobin levels, suggesting the possibility of thalassemia minor, while others have had splenomegaly of unknown cause. If these patients are excluded from consideration, there remain persons in whom no other disorder is manifest; some of these individuals have been shown to be carriers of abnormal hemoglobins.

The first description of an association between polycythemia and a hemoglobinopathy was that of Huisman, van Vliet, and Sebens (4) in 1958. Studying the Texel breed of Dutch sheep, they noted that animals with type II hemoglobin had higher total hemoglobin concentrations than animals with type I, and that hybrids (type I/II) had intermediate levels. The oxygen affinity of hemoglobin II was higher than that of hemoglobin I, and the authors suggested that "... oxygen, which is present in the blood, is less available to the tissues, resulting in an anoxia. Consequently, an increase of the total hemoglobin content of the blood may occur." A comparable disorder was encountered in man with the discovery of hemoglobin Chesapeake, an abnormal hemoglobin with increased oxygen affinity causing erythrocytosis in the heterozygous carriers within a family (5, 6). The pathogenesis of the human disorder appears to be exactly the same as in sheep. Polycythemia has now been found in association with several abnormal hemoglobins (3, 7–12) but homozygotes have not been described.

Some characteristics of patients, and of their hemoglobins, are summarized in Table I. Affected individuals show no stigmata of their disorder other than rubor. A few, like the carrier of hemoglobin Chesapeake shown in Figure 1, have survived to the eighth or ninth decade. At least one carrier was told, when donating to the Red Cross, that his blood was "as thick as that of someone living on top of a mountain." He was, essentially, and many of these patients show similarities to persons acclimatized to high altitude.

Detailed studies of erythropoietin excretion have been carried out in carriers of hemoglobins Yakima and Rainier (15). Excretion was normal when pa-

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TABLE I

Hemoglobins with Increased Oxygen Affinity which are Associated with Polycythemia

Hemoglobin	Reference	Hematocrit Values of Carriers (%)	Amino Acid Substitution	Percent of Abnormal Hemoglobin in Hemolysate	n	Bohr Effect
Chesapeake	5, 6	48–58	αFG4 arg → leu	30	1.3	Present
Yakima	7, 8	45–55	βG1 asp → his	38	1.0	Present
Kempsey	9	52–64	βG1 asp → asn	46	1.1	Present
Rainier	10	57–59	βH23 tyr → his	30	1.2	—
Hiroshima	11, 12	45–54	βH21 his → asp	50	2.3	Decreased
Ypsilanti	3	42–63		—	—	—

Amino acid substitutions have been located according to their structural rather than their sequential position (13, 14). Helical regions are assigned letters A through H, while inter-helical regions are assigned two letters: αFG4 is the fourth residue in the segment of the α chain between the F and G helices. Magnitude of heme-heme interaction (n), and variation in oxygen affinity with change in pH (Bohr effect), refer to purified solutions of hemoglobins Chesapeake, Yakima, and Hiroshima, and mixed hemolysates containing hemoglobins Kempsey and Ranier. In purified solutions of normal hemoglobin, n = 2.6–3.0, and a Bohr effect is present.

tients had their usual high hematocrit values; when phlebotomized to *normal* hematocrit values, erythropoietin excretion rose to supernormal levels. Some sensor, presumably in the kidney, appeared to be responding to the decreased amount of oxygen which could be delivered at normal tissue oxygen tension.

Not all hemoglobins which exhibit increased affinity for oxygen are associated with polycythemia (Table II). For polycythemia to be produced, the abnormal hemoglobin must comprise a significant proportion of the total amount present, and it must have no other deleterious qualities. Hemoglobin Lepore is found in a proportion of about 12 percent: Oxygen affinity of a hemolysate was normal despite the abnormal properties of the isolated hemoglobin (17). Carriers of this hemoglobin are somewhat anemic, for they have a thalassemia-like syndrome.

A number of unstable hemoglobins have increased affinity for oxygen. These include Zürich (20), Gun Hill (22), St. Mary's, Köln (25), and Freiburg (38). Carriers have compensated hemolytic anemia. Erythropoiesis proceeds at an accelerated rate, despite a normal hemoglobin concentration, because of decreased release of oxygen to the tissues. In these instances, chronic hemolytic anemia prevents the development of polycythemia, and some other physiologic response must be evoked to deal with tissue hypoxia.

Responses of this type may be particularly evident during pregnancy. Normally, the blood of the fetus has a higher oxygen affinity than than that of the mother. If a carrier of hemoglobin Zürich ($\alpha_2\beta_2{}^{E7arg}$) becomes pregnant, the normal gradient of oxygen affinity across the placenta is abolished: Abnormal β chains have increased the affinity of maternal blood, but the affinity of fetal blood, which lacks β chains, is unchanged (21). Such pregnancies can be carried to term without difficulty (Fig. 2). Similar situations exist in the female

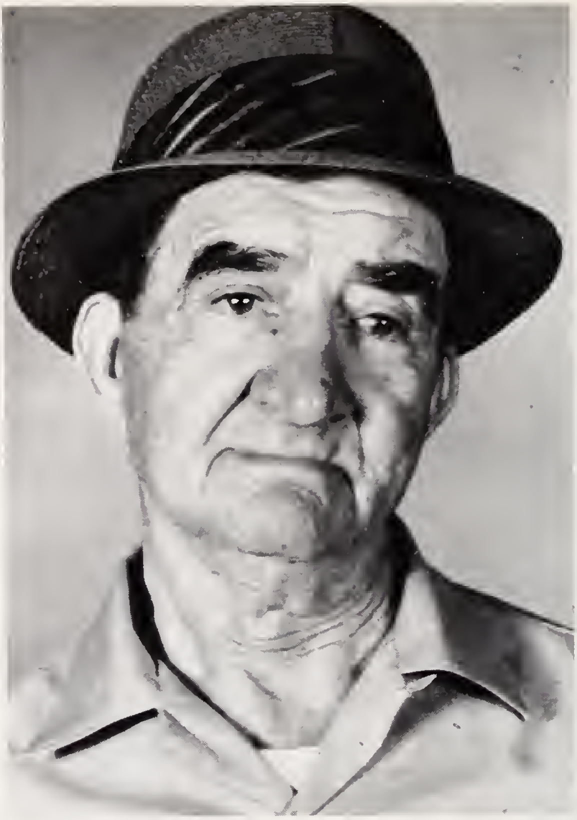

Fig. 1. An 86-year-old carrier of hemoglobin Chesapeake. An altered contact at the $\alpha_1\beta_2$ interface may be responsible for increased oxygen affinity of the abnormal hemoglobin: the patient shows no stigmata of the disorder other than rubor.

carrier of hemoglobin Chesapeake during pregnancy with a normal child (5), and in the pregnant llama (26). Alterations in placental blood flow, or diffusing capacity, may possibly be involved in these situations (8).

Mechanisms of Abnormal Oxygen Affinity

At a molecular level, greater understanding of the mechanisms of oxygen binding by normal hemoglobin has developed from studies of these mutants. The hemoglobin molecule exists in two different structural forms, the oxy- and the deoxy-configurations. Any alteration in amino acid composition which changes the stability of one form relative to the other, i.e., shifts the position of the equilibrium oxy-configuration $\rightleftharpoons$ deoxy-configuration, will alter oxygen affinity. The amino acid substitutions which produce increased oxygen affinity may do so by 1 of 4 complementary processes:

SUBSTITUTIONS IN THE IMMEDIATE ENVIRONMENT OF THE HEME GROUP

The first mechanism is exemplified by hemoglobin Zürich. The site of mutation is histidine E7(63)β, an amino acid residue normally contiguous to an

TABLE II

Hemoglobins with Increased Oxygen Affinity which are Not Associated with Polycythemia

Hemoglobin	Reference	Hematocrit Values of Carriers (%)	Amino Acid Substitution	Percent of Abnormal Hemoglobin in Hemolysate	n	Bohr Effect	Remark
Lepore	16, 17	30–42	Fusion of β and δ chains	12	2.6	Present	Oxygen affinity of hemolysate normal
J Capetown*	18, 19	—	αFG4 arg → gln	—	1.9	Present	Oxygen affinity of hemolysate increased
Zürich*	20, 21	35–46	βE7 his → arg	30	2.0	Present	Unstable
Gun Hill	22–24	45–48	Deletion βF9–FG4	30	1.0	Decreased	Unstable

Data for hemoglobin J Capetown refer to mixed hemolysates; data for hemoglobin Zürich refer to whole blood; and data for hemoglobins Lepore and Gun Hill refer to purified solutions of the abnormal hemoglobin.

* See ***Note added in proof*** (after References).

oxygen molecule bound to iron in the "heme pocket" (27). The substituted amino acid, arginine, does not fit into that cavity: It is thought to protrude outwards toward the surface of the molecule, resulting in the space remaining empty. Studies of the infra-red stretching frequency of carbonyl derivatives (28), and the nuclear magnetic resonance of the heme protons of cyanomet derivatives of hemoglobin Zürich (29), suggest that some change in the immediate environment of the heme group may have taken place. Altered affinity for oxygen may be a direct result of this change.

SUBSTITUTIONS AT SURFACES OF CONTACT BETWEEN SUBUNITS

A second mechanism by which structural alterations may affect oxygen affinity is applicable in more instances. These are cases in which the mutations are on surfaces of contact between molecular subunits. The hemoglobin molecule is composed of two pairs of polypeptide chains, arranged in a very precise sterochemical relationship. The α chains present different surfaces to each of the β chains: Contacts between these unlike chains are called $\alpha_1\beta_1$ and $\alpha_1\beta_2$. Contacts between like chains are called $\alpha_1\alpha_2$ and $\beta_1\beta_2$. Interactions across these surfaces not only hold the molecule together, but they may transmit influences from one heme group to another (14). Such heme-heme interactions are believed to produce the sigmoidal shape of the normal oxygen dissociation curve.

The substituted amino acids of hemoglobins Chesapeake, J Capetown, Yakima, and Kempsey are in positions which can alter contacts at the $\alpha_1\beta_2$ interface (27). Two hypotheses have been proposed to explain the effects of such alterations. The first of these takes the dimer ($\alpha\beta$) as the fundamental

FIG. 2. A carrier of hemoglobin Zürich, and her infant daughter. During pregnancy, the usual transplacental gradient of oxygen affinity was not present, but fetal oxygenation was maintained.

unit, and relates changes in function to changes in degree of aggregation of oxygenated and deoxygenated dimers, and the formation of highly reactive intermediate forms (30–33). Experimentally, altered states of aggregation have been demonstrated for hemoglobin Chesapeake (34), and for hemoglobin Kansas, a hemoglobin with low oxygen affinity (34, 35).

The second hypothesis takes the tetramer ($\alpha_2\beta_2$) as the basic functional unit (14), and relates increased oxygen affinity and decreased heme-heme interaction to altered transmission of forces from one subunit to the other. According to this point of view, loss of hydrogen bonds in hemoglobin Kansas, when in the oxy-configuration, increases the relative stability of the deoxy-configuration, and decreased oxygen affinity results (27). A shift in the position of equilibrium between the two configurations of hemoglobin Chesapeake, but in the opposite direction, could account for increased oxygen affinity of that molecule. Recently, a possible test of the dimer and tetramer hypotheses has been proposed, based upon observations of the properties of chemically modified hemoglobins (36).

SUBSTITUTIONS AT THE CARBOXY-TERMINUS OF THE β CHAIN

A third mechanism, involving structural alterations near the carboxy-terminus of the β chain, is exemplified by hemoglobins Rainier and Hiroshima. Recent studies suggest that this region is intimately involved in the Bohr effect (changes in oxygen affinity produced by changes in pH), and perhaps also in structural transitions between the two configurations of the molecule (36, 37). Interference with these functions may be responsible for the observed alterations in oxygen affinity.

SUBSTITUTIONS IN OTHER POSITIONS

Since a localized structural change can have long range effects throughout the entire molecule, substitutions at sites other than those listed above may alter the position of equilibrium between the oxy- and deoxy-configurations. Hemoglobin Freiburg may represent such a mutation (38).

Diagnostic Implications

The amino acid substitutions which have been found in these hemoglobins usually involve changes in electric charge. Since molecular size and shape are as important as polarity in mediating the effects described here, there is no *a priori* reason to expect all hemoglobins with altered oxygen affinity to exhibit altered electrophoretic mobility. The charge substitutions described here make identification easy, but they are not a necessary condition.

The clinical implications of that statement are clear: Persons with familial polycythemia may have abnormal hemoglobins although none can be demonstrated by electrophoresis. Oxygen affinity of the blood must be measured in such individuals, but the instruments involved are temperamental, to say the least. Spectrophotometric determinations seem to offer the most promise of a technique suitable for clinical use, and at least one such method has been devised (25). There are clearly some cases of familial polycythemia not caused by abnormal hemoglobins, but it seems likely that many patients may, indeed, have a hemoglobinopathy.

Acknowledgments

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* ***Note added in proof:*** Since this manuscript was submitted for publication, two important papers have appeared. Jenkins et al have described a second family possessing hemoglobin J Capetown in S Afr Med J 42:1151, 1968. Heterozygotes were slightly polycythemic, and their blood contained 35–48 percent of the abnormal hemoglobin. It would appear that the pathophysiologic effects produced by hemoglobin J Capetown are entirely analagous to those produced by hemoglobin Chesapeake.

Winterhalter et al have described the properties of purified hemoglobin Zürich in Europ J Biochem 11:435, 1969.

A Reappraisal of the "Myeloproliferative Disease" Concept*

HARRIET S. GILBERT, M.D.

The concept of the "myeloproliferative disorders" was developed during the first half of the twentieth century to describe a group of clinical syndromes which were believed to represent varied manifestations of an underlying abnormality of the primitive mesenchymal precursor cell from which hematopoietic tissue and its supporting stroma are derived. The theory that such apparently dissimilar syndromes as polycythemia vera, agnogenic myeloid metaplasia, essential thrombocythemia, erythroleukemia, and chronic myelocytic leukemia are variants of the same underlying disorder has dominated our approach to these diseases for the past two decades, a period in medical history during which dramatic advances in technology have permitted new insights into cell biology. The purpose of this paper is to review the evolution of the concept of "myeloproliferative disease" and to consider whether this working hypothesis, when examined in the light of recent advances, is still a useful one in dealing with this group of diseases.

All of the syndromes included in the category of "myeloproliferative disease" have a similar history. The earliest reports of each focussed on the one most striking finding in the peripheral blood or bone marrow, and each disorder was described first as a specific disease entity characterized by increased proliferation of a single cell type. Even at this time the simultaneous involvement of more than one hematic cell population was noted, but no explanation for this finding was offered. As more cases were examined, difficulties in assigning certain patients to any one of the disease categories previously described were encountered. A patient with markedly increased erythrocytes, granulocytes, and platelets who had a large spleen defied classification under the existing nosologic system. The penchant for defining new disease entities which flourished at this time led to the description of still more diseases in order to provide diagnostic categories for almost all possible permutations of hematic cell proliferation that might occur. The literature mushroomed with redundancies such as chronic polycythemia with enlarged spleen (1), splenomegalic or myelopathic polycythemia (2), generalized osteosclerosis with chronic polycythemia (3), chronic nonleukemic myelosis (4), megakaryocytic myelosis (5), myeloid megakaryocytic hepatosplenomegaly (6), and leukoerythroblastic anemia (7).

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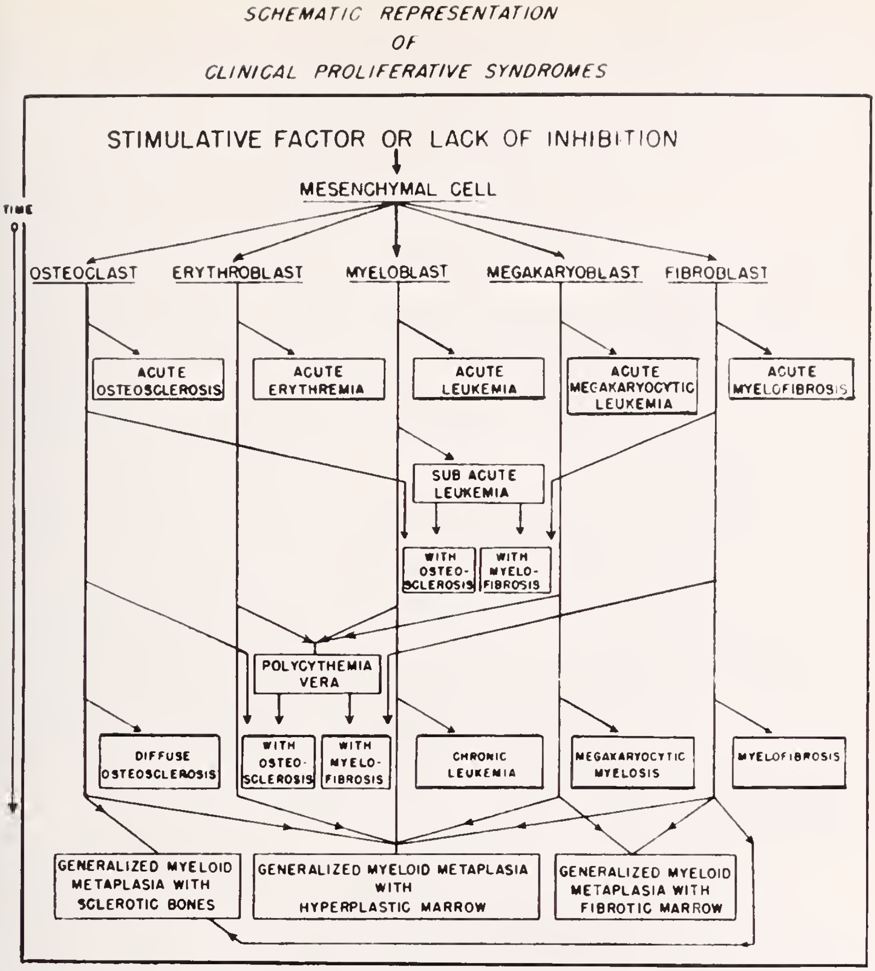

SCHEMATIC REPRESENTATION
OF
CLINICAL PROLIFERATIVE SYNDROMES
STIMULATIVE FACTOR OR LACK OF INHIBITION
MESENCHYMAL CELL
TIME
OSTEOCLAST
ERYTHROBLAST
MYELOBLAST
MEGAKARYOBLAST
FIBROBLAST
ACUTE OSTEOSCLEROSIS
ACUTE ERYTHREMIA
ACUTE LEUKEMIA
ACUTE MEGAKARYOCYTIC LEUKEMIA
ACUTE MYELOFIBROSIS
SUB ACUTE LEUKEMIA
WITH OSTEO-SCLEROSIS
WITH MYELO-FIBROSIS
POLYCYTHEMIA VERA
DIFFUSE OSTEOSCLEROSIS
WITH OSTEO-SCLEROSIS
WITH MYELO-FIBROSIS
CHRONIC LEUKEMIA
MEGAKARYOCYTIC MYELOSIS
MYELOFIBROSIS
GENERALIZED MYELOID METAPLASIA WITH SCLEROTIC BONES
GENERALIZED MYELOID METAPLASIA WITH HYPERPLASTIC MARROW
GENERALIZED MYELOID METAPLASIA WITH FIBROTIC MARROW

FIG. 1. See text (reprinted by permission from Wasserman, L. R.: Polycythemia Vera—Its Course and Treatment: Relation to Myeloid Metaplasia and Leukemia. Bull NY Acad Med 3:343, 1954).

Although this multiplicity of nosologic entities provided the physician with a diagnostic label for his patient, often, as single patients were observed over a period of years, the clinical status was seen to change, necessitating a new diagnosis. It was the observation of these transitions that provided the greatest impetus to the development of a unifying theory. Minot and Buckman (8), in 1923, commented that "there occur cases of erythremia that illustrate multiple varieties of varying degrees of primary pathological activity of myeloid tissue which usually conforms to a definite type." Vaughan and Harrison (9) suggested, in 1939, that polycythemia vera, megakaryocytic leukemia, and myelosclerosis with leukoerythroblastic anemia form a group of closely related conditions, and that "the evidence available suggests that the cases so described may be regarded as examples of the same underlying dysplasia." Nathan Rosenthal and Frank Bassen (10) (1938) called attention to the "peculiar and intricate course" of polycythemia vera, stating that "symptomatic and hematologic changes may continue until death and these may become clarified only when the function of the bone marrow is understood; for primary polycythemia is apparently a disease of the entire bone marrow, not merely of the red blood cell elements." In 1948, Eli Moschcowitz (11) discussed the biology of polycythemia vera and leukemia and implied that the transitions observed during the course of polycythemia vera were understandable if the multipotentiality of the cell from which the marrow originated was accepted. He cited Klemperer's (12) tenet that cell morphology is not absolute and true to type, but is relative and depends upon the potentialities of the cytoplasmic reticulum of the myeloid and lymphoid tissue which, under various abnormal conditions, reverts to its embryonic functions.

The various ideas which had been put forth over the years to establish order out of the chaotic case material were synthesized by Martin Rosenthal (13) in 1950, when he published a schematic representation of clinical proliferative syndromes (Fig. 1), and stated that "involvement of the multipotential mesenchymal cell offers an attractive explanation for the puzzling and frequent interassociation of polycythemia vera, leukemia, osteosclerosis, myelofibrosis, and thrombocytosis. Subtle changes occurring with the passage of time, in the types of cells derived from the mesenchymal cell may result in different clinical pictures, all nonetheless showing the basic fault of abnormal cell proliferation."

Dameshek (14) coined the term "myeloproliferative disorders" for these

TABLE I

The Myeloproliferative Disorders

Syndromes	Myelostimulatory Factors				
	Bone Marrow				Potential Bone Marrow
	Erythroblasts	Granulocytes	Megakaryocytes	Fibroblasts	Myeloid Metaplasia of Spleen and Liver
Chronic granulocytic leukemia	±	+++	+ to +++	+	++
Polycythemia vera	+++	++	++ to +++	+ to +++	+ to +++
Idiopathic or agnogenic myeloid metaplasia of spleen	±	±	+++	+ to +++	+++
Megakaryocytic leukemia	±	±	+++	+	+ to +++
Erythroleukemia (including diGuglielmo syndrome)	+++	+	±	±	+ to +++

Degrees of proliferation: + = slight; ++ = moderate; +++ = marked.

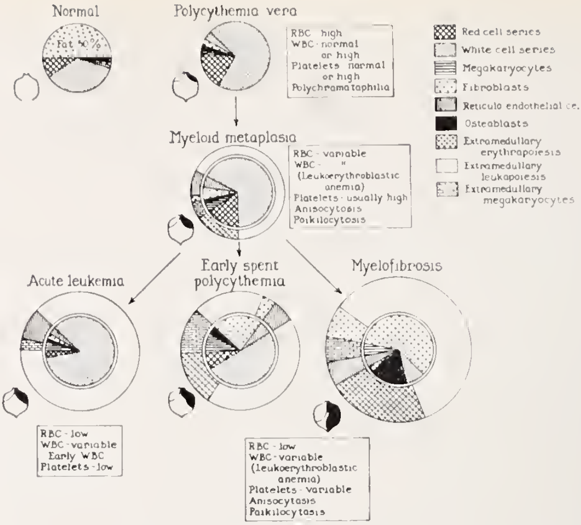
Reprinted by permission from Dameshek, W.: Some Speculation on the Myeloproliferative Syndromes, Blood 6:372, 1951.

closely related conditions in 1951, and broadened the concept further by including erythroleukemia (Table I). In 1954, Wasserman (15) reported his findings in a large series of cases of polycythemia vera, and presented a hypothetical concept of the course of this disease, demonstrating that it is part of a reticulosis with an initial manifestation in erythrogenetic activity and simultaneous or subsequent involvement of other hematic and non-hematic mesenchymal derivatives (Fig. 2).

The "myeloproliferative disease" concept is predicated upon the existence of multipotential hematopoietic precursor cells that are capable of giving rise to a trilineage of cells, those of the erythroid, myeloid, and megakaryocytic series. At the time of its evolution, the existence of such precursor cells was, itself, an hypothesis. The recent advances of greatest significance in support of the concept deal with the demonstration of pluripotential stem cells. In 1961, Till and McCulloch (16) reported that in normal mouse hematopoietic tissue there is a class of cells which, when transplanted into heavily irradiated mice, can proliferate and form macroscopic colonies. These colonies appear as discrete clusters of hematopoietic cells in the recipient's spleen. Microscopic analysis of single colonies shows that differentiation into cells of the erythrocytic, granulocytic, and megakaryocytic series occurs. Becker, McCulloch, and Till (17) exploited this technique further by irradiating the donor marrow sufficiently to produce chromosome abnormalities, reasoning that if a spleen colony develops from one cell, then all the cells of a colony arising from an atypical cell would bear the abnormal karyotype. If, on the other hand, a spleen colony derives from an undispersed aggregate of two or more injected cells, a mixed karyotype should be present within a single colony. They found that when recognizable marker chromosomes were present in the cells of a colony, an overwhelming majority of the cells within that colony contained the markers.

The same workers had previously shown that a linear curve relates the number of nucleated marrow cells that are transplanted to the number of colonies that develop in the spleen without an initial threshold, and that the radiation survival curve of cells able to form colonies closely resembles the survival curves obtained for single cells in cell culture or tumor transplants. Their cytological evidence, taken in conjunction with these observations, supports the view that spleen colonies represent clones of cells derived from single multipotential hematopoietic stem cells.

The development of a technique for karyotyping human cells, and the discovery of a defect associated with chronic myelocytic leukemia (the Philadelphia chromosome, Ph^1) (18) has produced supportive evidence for the existence of a common ancestry of the erythroid and granulocytic cell series in humans. Indirect evidence for the presence of the Ph^1 chromosome in erythroid as well as myeloid precursors was first presented by Whang et al (19), when they observed a 90 to 100% incidence of the abnormal chromosome in bone marrow cells of 13 patients in drug-induced clinical remission who were known to have a high proportion of dividing normo-


Normal
Fat 50%
Polycythemia vera
RBC high
WBC normal or high
Platelets normal or high
Polychromatophilia
Red cell series
White cell series
Megakaryocytes
Fibroblasts
Reticulo endothelial cells
Osteablasts
Extramedullary erythropoiesis
Extramedullary leukopoiesis
Extramedullary megakaryocytes
Myeloid metaplasia
RBC - variable
WBC - "
(Leukoerythroblastic anemia)
Platelets - usually high
Anisocytosis
Poikilocytosis
Acute leukemia
Early spent polycythemia
Myelofibrosis
RBC - low
WBC - variable
Early WBC
Platelets - low
RBC - low
WBC - variable
(leukoerythroblastic anemia)
Platelets - variable
Anisocytosis
Poikilocytosis

Hypothetical concept of the course of polycythemia vera. The blood, bone marrow and extra-osseous potential marrow, considered "the blood tissue," reflect simultaneous changes occurring during the course of polycythemia vera. In polycythemia vera the hyperplastic marrow expands to encompass the total marrow space at the expense of the fat cells. There is a panmyelosis with stimulation of all hematic and nonhematic cellular derivatives of the reticulum cell. Blood examination reveals an erythrocytosis and usually a granulocytic leukocytosis, thrombocytosis and immature red and white cells. Splenomegaly and hepatomegaly are frequently present, initially due to the increase in blood volume but subsequently associated with reticulum cell hyperplasia and heteroplastic blood formation. With continued hyperactivity the dormant extra-osseous potential marrow becomes functionally active and the site of extramedullary hematopoiesis (stage of myeloid metaplasia) as the spleen and liver enlarge (outer circle designates extramedullary blood formation). The bone marrow shows diminution in erythrogenesis and proliferation of reticulum cells, fibroblasts, megakaryocytes and white cells. The peripheral blood mirrors the marrow changes with usually a mild to moderate anemia although normal values (compensated state) may prevail for many years. Thrombocytosis is common as is a leuko-erythroblastic blood picture. With time, progressive changes in cellular composition of the marrow occur (stage of early spent polycythemia). Fibroblastic or osteoblastic proliferation encroaches on hematic cell activity with a reduction particularly in erythropoiesis. Megakaryocytes appear more plentiful due either to increased proliferative activity or a quantitative decrease in other hematic cells. The liver and spleen, the site of extramedullary hematopoiesis, are not encased in bone, hence can expand markedly. There is now a leukocytosis and thrombocytopenia or thrombocytosis, leuko-erythroblastic anemia with morphologically bizarre red cells. Further fibroblastic or osteoblastic proliferation may obliterate the marrow cavity with all blood formation occurring in the extra-osseous marrow (stage of myelofibrosis). Note that nonhematic cell proliferation occurs in the liver and spleen also. The anemia becomes more severe with usually a leukopenia, thrombopenia and a more marked leuko-erythroblastic blood picture. Hepatosplenomegaly may be tremendous. This phase of myelofibrosis (myeloid metaplasia) may be a terminal picture in polycythemia vera. Other lines of proliferation may gain ascendency, e.g., the white cells, with development of an acute leukemia occasionally preceded by a stage similar to chronic myelocytic leukemia; megakaryocyte hyperactivity may result in so-called megakaryocytic myelosis, etc.

Acute leukemia, spent polycythemia and myelofibrosis are all depicted as stemming from a common stage of myeloid metaplasia. Variation in proliferative activity of any combination of cellular categories may produce complex clinical and pathological syndromes. Thus, well developed myelofibrosis may frequently show large numbers of myeloblasts and myelocytes in the blood and, similarly, a terminal acute blastic leukemia may show extensive fibroblastic proliferation. (The area represented for any specific cellular series is only approximate and merely represents an attempt to demonstrate graphically a few of the multiple developmental potentialities in polycythemia vera.)

FIG. 2. See text (reprinted by permission from Rosenthal, M. C.: Extramedullary Hematopoiesis; Myeloid Metaplasia, Bull New Eng Med Cent 12:154, 1950).

blasts in the marrow. More direct evidence was obtained by Clein and Flemans (20), who combined cytogenetic and staining techniques to demonstrate siderotic granules in the cytoplasm of some Ph¹-positive cells. Rastrick, Fitzgerald, and Gunz (21) made chromosome preparations of bone marrow from a patient with chronic granulocytic leukemia after the cells had been cultured with radioactive iron. Under the conditions of the experiment, in which only the erythroid cell series was labelled, autoradiograms showed that the labelled cells in metaphase contained the Ph¹ chromosome. The origin of megakaryocytes from the same precursor as the erythroid and granulocytic series in human marrow is suggested strongly by the presence of the Philadelphia chromosome in polyploid marrow cells which appear to be megakaryocytes, but the evidence remains circumstantial (19).

The technique of chromosome analysis has provided the most convincing evidence for the existence of a pluripotent precursor cell in human bone marrow, thus strengthening the validity of the "myeloproliferative disease" concept. Ironically, it is the results of chromosome analyses within this group of diseases which have cast some doubt on the validity of regarding them as a spectrum of interrelated conditions. Since 1960, when the Philadelphia chromosome was described, karyotyping has been performed on large numbers of patients with various forms of "myeloproliferative disease" (22–26). Although a variety of other karyotypic abnormalities have been found in polycythemia vera, myelofibrosis, and myeloid metaplasia, no consistent pattern has emerged, whereas the presence of the Philadelphia chromosome has invariably been associated with chronic myelocytic leukemia. Reports of the presence of a Ph¹ chromosome in cases considered as myelofibrosis-myeloid metaplasia do not detract from the specificity of this finding, since fibrosis may occur in otherwise typical chronic myelocytic leukemia (27), and the distinction between chronic myelocytic leukemia and myelofibrosis-myeloid metaplasia may be difficult to make on either clinical or laboratory grounds.

The involvement of granulocytes in the proliferative disturbance of polycythemia vera and the frequent transition of polycythemia vera into acute myeloblastic leukemia have been taken as strong evidence in favor of its close etiologic relationship to chronic granulocytic leukemia. In support of this theory, one would expect that abnormalities of granulocytes present in either of these variants would be shared by both. This, in fact, has not been the case. As increasing numbers of patients with polycythemia vera have been studied, in the stage of myeloid metaplasia-myelofibrosis as well as during the erythremic phase, the rarity of Ph¹-positivity is impressive. Aside from a karyotypic abnormality, originally thought to be a Philadelphia chromosome when first reported in two brothers with well-documented polycythemia vera (28), but which, on reexamination was a small familial "Y" chromosome (29), the case of Kemp, Stafford, and Tanner (30) remains the only reported instance of this association. The patient was a 39-year-old male in whom a hematocrit of 59% was discovered during treatment (medication not specified) for chronic eczema. The initial white blood count was 25,200/mm³, and the platelet count was 486,000/mm³. Splenomegaly and hepatomegaly were absent. The first determinations of leukocyte alkaline phosphatase were performed and found to be low following three treatments with radioactive phosphorus. At that time a Philadelphia chromosome was identified. Seven months later the patient developed a picture of chronic myelocytic leukemia with splenomegaly and leukocytosis of 132,000/mm^3, which terminated in acute myeloblastic leukemia in the ensuing 3 months. The diagnosis of polycythemia vera in this case can only be presumptive, since erythrocytosis was not documented by means of blood volume studies, the highest hematocrit was only 59% before treatment, and splenomegaly was absent. Unfortunately, since chromosome and enzyme studies were not obtained prior to treatment, there is no documentation of a change in either of these parameters during the patient's course. If the change in clinical status is accepted as representing a transition, this is the only case on record that demonstrates the typical cytogenetic and enzyme changes of chronic myelocytic leukemia occurring in the course of polycythemia vera.

Advances in characterization of cellular enzymes have equipped us with still another means of studying the "myeloproliferative syndromes." Here, again, the finding of reduced or absent leukocyte alkaline phosphatase activity (LAPA) in 92 percent of untreated patients with chronic granulocytic leukemia, in contrast to an elevated score in 91 percent of patients with untreated polycythemia vera (31), provides a distinct difference between the two disease states. The effect of treatment on levels of this enzyme also differs, a rise in LAPA to normal levels being seen frequently in chronic granulocytic leukemia, as contrasted with unaltered enzyme levels throughout the course of polycythemia vera. In view of the extremely wide range of scores reported in normal subjects and the vagaries inherent in the technique of LAPA determination, reports of low scores in polycythemia vera or high scores in chronic myelocytic leukemia found in the course of survey studies are not as useful in interpreting the relationship of these diseases as serial studies performed during the entire course of the disease would be. In the cases where this has been done, the evolution from polycythemia vera with high LAPA to chronic myelocytic leukemia with reduced LAPA has not been observed (32, 33).

On the basis of genetic and biochemical studies of the clinical variants that comprise the "myeloproliferative disorders," a distinctive picture has emerged for chronic myelocytic leukemia. The continuing failure to demonstrate the Philadelphia chromosome in polycythemia vera during its various stages, the differences in levels and behaviour of LAPA, and the failure to observe transitions in the karyotype or enzyme picture despite apparent clinical transition from polycythemia vera to a picture resembling chronic myelocytic leukemia, tend to polarize at least two of the members of the "myeloproliferative syndromes," chronic myelocytic leukemia being set apart from polycythemia vera. The remaining entities included in the group would appear to segregate with polycythemia vera on the basis of current knowledge.

Whether the constellation of abnormalities observed in chronic myelocytic leukemia has any relevance to the etiologic mechanism(s) operative in this disease, and whether the "myeloproliferative syndromes" result from several causes and are related only by virtue of the limited ways in which the primitive precursor cell can react to a stimulus, are questions that hopefully

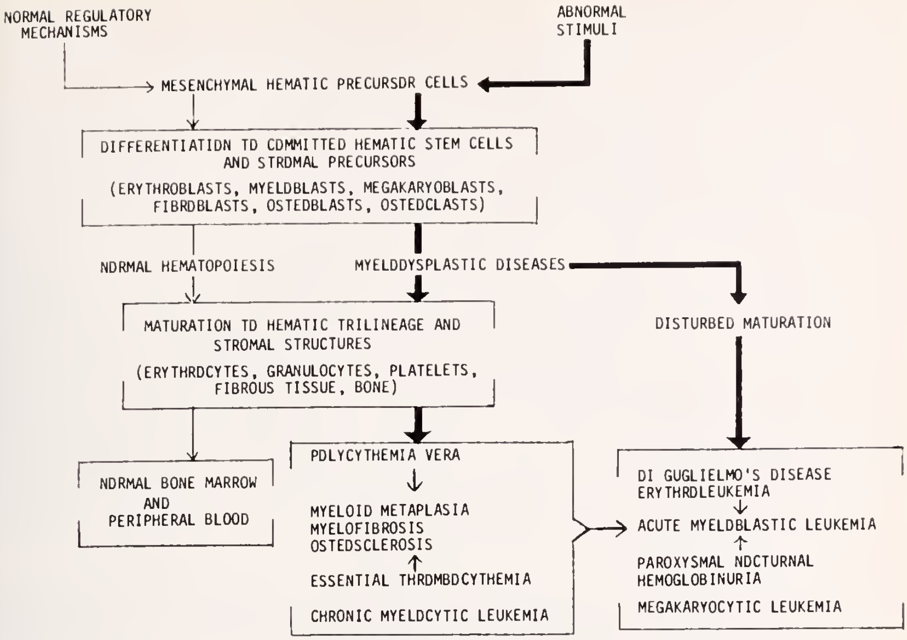

NORMAL REGULATORY MECHANISMS
ABNORMAL STIMULI
MESENCHYMAL HEMATIC PRECURSOR CELLS
DIFFERENTIATION TO COMMITTED HEMATIC STEM CELLS AND STROMAL PRECURSORS
(ERYTHROBLASTS, MYELOBLASTS, MEGAKARYOBLASTS, FIBROBLASTS, OSTEOBLASTS, OSTEOCLASTS)
NORMAL HEMATOPOIESIS
MYELODYSPLASTIC DISEASES
MATURATION TO HEMATIC TRILINEAGE AND STROMAL STRUCTURES
(ERYTHROCYTES, GRANULOCYTES, PLATELETS, FIBROUS TISSUE, BONE)
DISTURBED MATURATION
NORMAL BONE MARROW AND PERIPHERAL BLOOD
POLYCYTHEMIA VERA
MYELOID METAPLASIA
MYELOFIBROSIS
OSTEOSCLEROSIS
ESSENTIAL THROMBOCYTHEMIA
CHRONIC MYELOCYTIC LEUKEMIA
DI GUGLIELMO'S DISEASE
ERYTHROLEUKEMIA
ACUTE MYELOBLASTIC LEUKEMIA
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
MEGAKARYOCYTIC LEUKEMIA

FIG. 3. Schema for the "myelodysplastic diseases:" The integrity of bone marrow depends upon differentiation of mesenchymal hematic precursor cells into committed stem cells that then undergo maturation, giving rise to a trilineage of hematic cells and the supporting stroma of the bone marrow. Complex regulatory mechanisms determine the rate of differentiation and maturation and the balance between the various cell populations. Abnormal stimuli which act upon the mesenchymal precursor cell or interfere with normal regulatory mechanisms controlling these cells may alter the normal pattern of hematopoiesis and produce abnormal growth of the precursor cell or "myelodysplastic disease."

In some cases the dysplasia is manifested primarily as an increase in hematopoietic activity at intra- and extra-medullary sites, with preservation of maturation and production of mature cell populations. In other cases, the dysplasia is characterized by impairment of maturation, resulting in the appearance of immature cell populations or in failure of production of functioning cell lines due to ineffective hematopoiesis.

That an abnormal stimulus may be expressed in a variety of ways is seen by the transitions from one clinical expression to another during the course of certain of these diseases. However, the appearance of common clinical expressions does not necessarily imply a common etiology for the "myelodysplastic diseases," but may simply represent the limited ways in which the primitive precursor cell reacts to varying stimuli.

will continue to motivate research during the latter part of the twentieth century.

Sir Frances Lewis' observation that "The continued separation of disease into types can be pressed too far, and a subdivision which may perhaps have some immediate practical use can help to conceal underlying mechanisms held in common and thus to hinder progress in studying disease," has been useful when applied to "myeloproliferative disease" in that it has brought us to the point where the preceding questions are recognized as requiring answers. At this stage in our knowledge, it may be that the practice of regarding all of the diseases with similar clinical pictures as one syndrome tends to obscure the underlying mechanisms by ignoring or discounting the differences that have been uncovered. Perhaps the time has come to revise not only the approach to the "myeloproliferative diseases" but even the term, which may have outgrown its application. The trend toward broadening the concept of "myeloproliferative disease" to include DiGuglielmo's disease and, as suggested recently by Dameshek (34), paroxysmal nocturnal hemoglobinuria, derives from the idea that all of these clinical entities are diseases of the pluripotent marrow precursor cell. "Proliferative" ("to grow by rapid production of new parts, cells, buds or offspring") may be a misnomer for the events observed in this group of diseases, particularly if its newer members are to be accommodated. "Myelodysplasia," or "myelodysplastic diseases," is suggested as a general descriptive term that is more in keeping with the concept of a group of diseases characterized by abnormal growth of the primitive precursor cell (Fig. 3). While of some use in providing a frame of reference and flexibility in the interpretation of clinical phenomena, any term employed to group these diseases should be used with the understanding that conclusions regarding interrelationships or underlying etiology are premature.

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Effect of Chloroquine on the Fine Structure of Lymphocytes Transformed with Phytomitogens *in vitro**

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The *in vitro* stimulation of human peripheral blood lymphocytes with phytomitogens initiates a series of biochemical and morphological events which lead to cellular transformation and mitotic activity. The mechanism whereby phytomitogens induce these alterations in metabolic activity has not been delineated. The "resting" lymphocyte has been shown to contain a small number of lysosomes (3), and recent studies have suggested that lymphocyte lysosomes may participate in the initial phases of cell stimulation (13, 14). Within 30 minutes to 2 hours after exposure to phytohemagglutinin (PHA) or pokeweed mitogen (PWM), there is a release of acid hydrolases (e.g., acid phosphatase and beta glucuronidase) into the cytoplasm, followed by, or concurrent with, an increase in the rate of synthesis of RNA and protein (4, 13, 14). Recently, several pharmacologic agents have been shown to stabilize lysosomal membranes and to inhibit the *in vitro* lymphocyte response to phytohemagglutinin (13, 15). Changes in lysosome membrane permeability may be a major requisite for anti-inflammatory action of such drugs (19). One of these, chloroquine, 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline, has been studied extensively and has been shown to affect lymphocytes as well as other cell types *in vivo* and *in vitro* (1, 9–12). Studies of leukocytes from patients with sarcoidosis treated with chloroquine have shown large toxic granules in leukocytes (9) and similar granules have been observed in the intraerythrocytic forms of malaria parasites following the administration of chloroquine (2, 16–18). *In vitro* study of the pharmacologic properties of this drug has yielded similar results, and the phenomenon has been interpreted as being related to increased autophagy (10–12). The precise effects of chloroquine on cells have not been delineated. Accordingly, a study of the fine structural changes following exposure to chloroquine was undertaken in an attempt to further elucidate the site of action of chloroquine on lymphocytes stimulated with phytomitogens

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and to provide additional evidence concerning the mechanism of stimulation of lymphocytes by plant mitogens.

Materials and Methods

PREPARATION OF LYMPHOCYTE CULTURES

Peripheral blood lymphocytes, obtained from three normal adult volunteers, were sedimented by gravity at room temperature, and separated using glass wool columns as described in detail elsewhere (8). The final cell suspension consisted of 92–97% small lymphocytes. The cultures contained aliquots of 2×10^6 lymphocytes in 3 ml Eagle's minimal essential spinner medium (MEM-S) containing 10% fetal calf serum, 0.02 M L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

The following mitogens were added in amounts previously found to yield optimal incorporation of thymidine-2-C^{14} at 72 hours (8): phytohemagglutinin (PHA-M, Difco Laboratories, Lot 497816), 500 μg per culture; pokeweed mitogen (PWM-TCA), 5 μg per culture; purified Concanavalin A (this purified material was kindly provided by Dr. Irvin E. Liener), 50 μg per culture; and purified wax bean glycoprotein (this purified material was kindly provided by Dr. Irvin E. Liener), 30 μg per culture. Chloroquine diphosphate (Aralen diphosphate, Sterling Winthrop Laboratories, Rensselaer, N.Y.) was added at concentrations from 0.1 μg to 50 μg per culture.

Triplicate cultures were assayed for ^{14}C-thymidine incorporation into DNA using 0.2 μc ^{14}C-thymidine per culture, specific activity 54 mC/mM (New England Nuclear Corporation, Boston, Mass.), added 8 hours prior to harvest. The scintillation techniques employed are described elsewhere (8).

ELECTRON MICROSCOPY

Cell preparations were centrifuged at 225 × g for 10 minutes and the pellets were fixed in 1.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2) containing 1% sucrose for 18–24 hours. Pellets were post-fixed in Millonig's phosphate-buffered osmium tetroxide, *en bloc* stained with 0.5% uranyl acetate in 50% ethanol, dehydrated, and embedded in araldite. Sections were prepared using an LKB-Ultrotome III, stained with lead citrate and placed on carbonized-collodion grids. The specimens were examined with either a Siemens Elmiskop I or Phillips 200 electron microscope.

Results

EFFECTS OF CHLOROQUINE ON ^{14}C-THYMIDINE INCORPORATION BY MITOGEN STIMULATED LYMPHOCYTES

Addition of chloroquine in amounts of less than 10 μg per culture had little or no effect on ^{14}C-thymidine incorporation by mitogen stimulated lymphocytes. At 30–50 μg per culture, there was almost complete inhibition of ^{14}C-thymidine incorporation. The ^{14}C-thymidine incorporation by lympho-

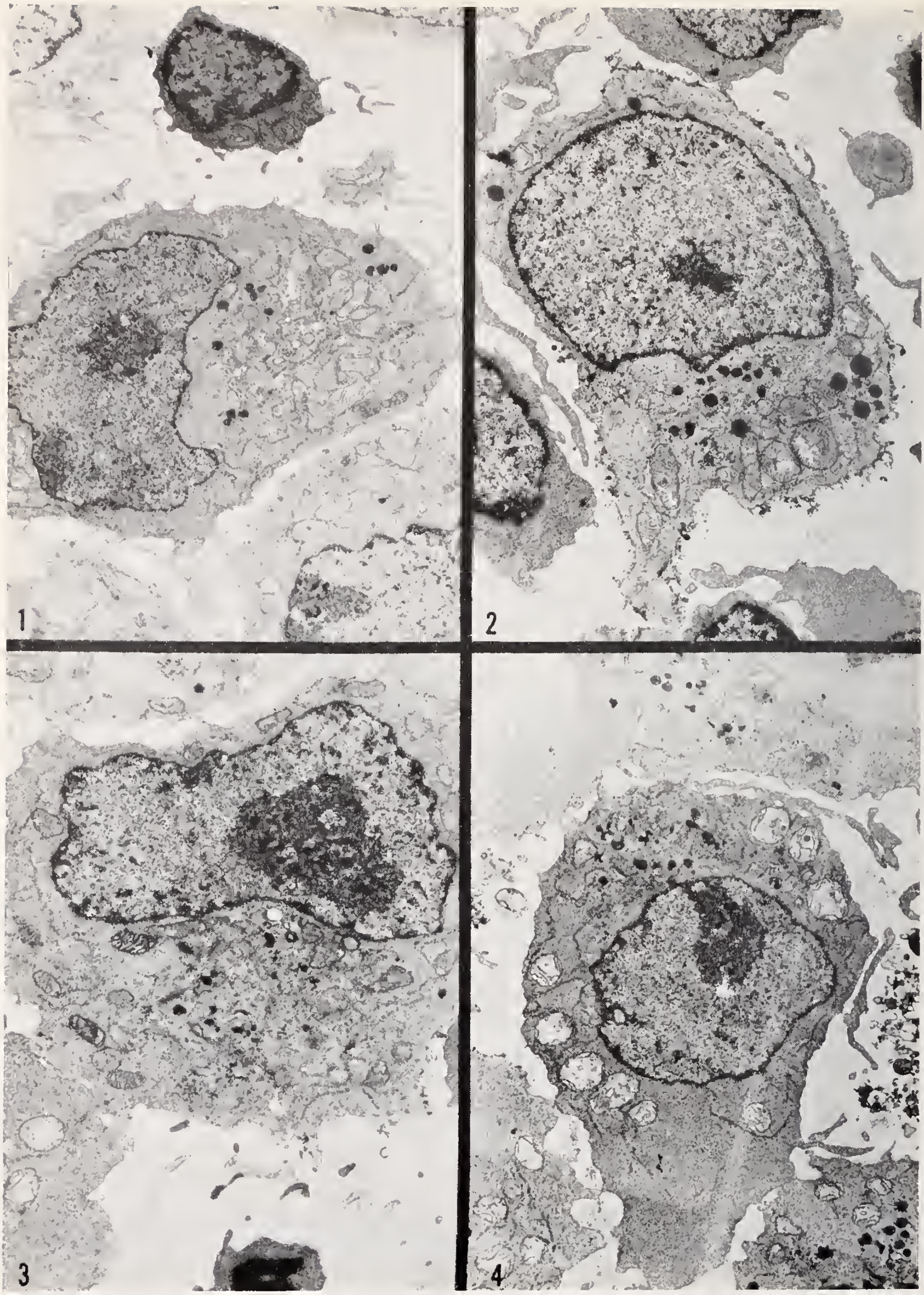

PLATE I

Blast-like cells from 72 hour lymphocyte cultures stimulated with various phytomitogens: Cells fixed with glutaraldehyde-osmium and *en bloc* stained with uranyl acetate. Grids stained with uranyl acetate-lead citrate.

Fig. 1. Cell from culture stimulated with pokeweed mitogen (PWM): Prominent

TABLE I

Effects of Chloroquine on the Incorporation of ^{14}C-Thymidine by Purified Lymphocytes Stimulated with Different Mitogens (after 72 Hours). Expressed as Counts per Minute.

	Phytohemagglutinin 500 µg/culture			Pokeweed Mitogen 5 µg/culture		Concanavalin A 50 µg/culture	Wax Bean Glycoprotein 30 µg/culture
	Donor #1	Donor #2	Donor #3	Donor #1	Donor #2	Donor #1	Donor #1
"Resting" cells	158	57	44	158	57	158	158
Lymphocyte response to mitogen (without chloroquine)	58,516	13,759	11,069	8,750	2,299	23,537	54,261
Chloroquine dose culture							
0.1 µg	41,692	—	—	—	—	20,632	—
1 µg	36,434	—	11,069	—	—	12,490	
10 µg	41,351	12,518	9,391	8,628	2,511	8,413	4,683
20 µg	—	—	1,764	—	—	—	—
30 µg	3,376	1,692	519	144	207		3,244
50 µg	86	137	22	34	10	10	53

cytes which received 50 µg chloroquine per culture was less than for "resting" lymphocytes with but one exception (Donor #2 PHA, Table I). Similar effects were observed with each of the mitogens studied: PHA, PWM, Concanavalin A, and wax bean glycoprotein. These results are shown in Table I.

FINE STRUCTURAL STUDIES

The fine structural features of phytomitogen stimulated lymphocytes in 72 hour cultures have been reported previously (5–7). Although there are subtle differences in the extent of organelle development, in general, mitogen transformed lymphocytes have similar morphologic features. These include euchromatic nuclei, large nucleoli, extensively developed Golgi zones, many electron-dense lysosome-like organelles, and numerous ribosomes (Plate I).

Cultures which received 50 µg chloroquine had few intact cells present at 72 hours, and contained only nuclear fragments and debris. At doses of 10–30 µg per culture, significant numbers of intact cells were observed (cell viability at 72 hours was assessed by the exclusion of 0.067% Erythrosin B. At these doses of chloroquine, 60–70 percent of cells were viable after 72 hours). Nuclear morphology was typical of that in transformed lymphocytes: predominantly, euchromatic nuclei with large nucleoli. Many of these cells contained large organelles with several types of substructure (Plates II and III). Occasional vacuoles were observed, which were contiguous with the

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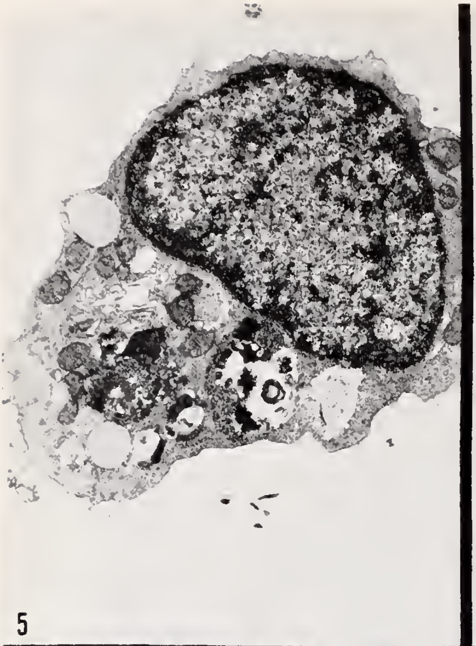
nucleolus and well-developed Golgi zone are evident. A small lymphocyte is present at the top center of the micrograph (× 5,500).

FIG. 2. Concanavalin A stimulated cell. Well developed Golgi zone containing several lysosomes and prominent microvilli (fimbriae) at cell membrane are evident (× 5,500).

FIG. 3. Blast-like cell from PWM culture (× 7,000).

FIG. 4. Blast-like cell from phytohemagglutinin (PHA) stimulated culture. Mitochondria show vacuolization (× 5,500).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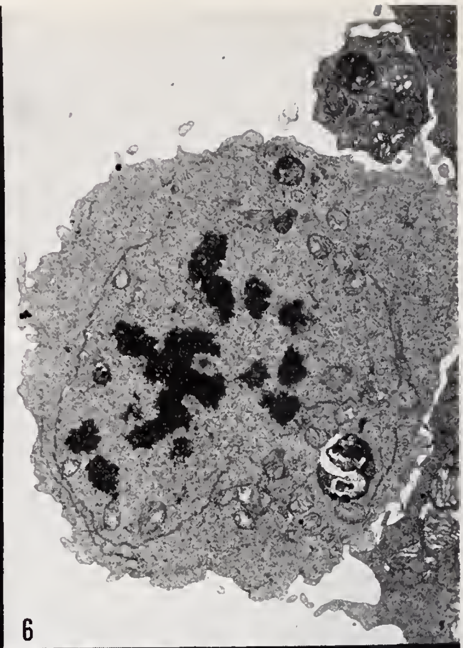
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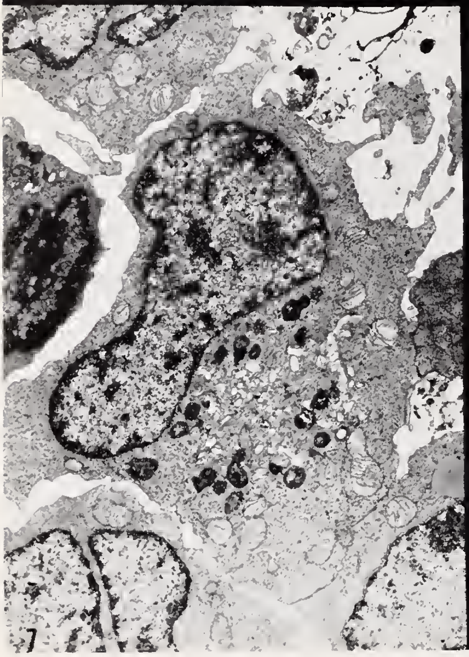
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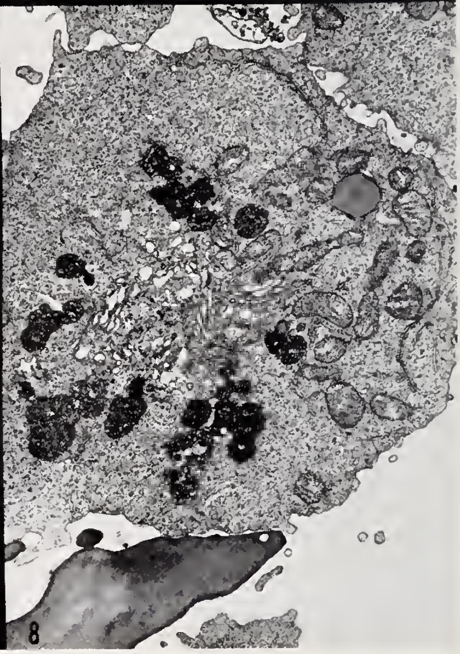
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PLATE II

Cells from 72 hour lymphocyte cultures stimulated with phytomitogens in presence of chloroquine.

Fig. 5. Cell from culture incubated with Concanavalin A and 10 μg chloroquine. Several large vacuolated structures, some of which contain myelin-like bodies are evident (× 9,100).

Fig. 6. Metaphase cell from culture incubated with PHA and 10 μg chloroquine. Large vesiculated organelle is present at the lower right (× 5,500).

cell membrane and contained debris, whereas others contained concentric whorls of electron-dense material (myelin figures) (Plate II, Fig. 5). The most frequently observed abnormality was the presence of single membrane-bound vesicular structures, usually originating in the Golgi zone (Plate II, Figs. 5, 7, and 8; Plate III). Structures of this type were also observed in mitotic cells (Plate II, Fig. 6). These structures varied from 0.2–1.3 microns in diameter. Similar structures were observed in cells stimulated with each of the mitogens studied. Fine structural alterations were not observed in unstimulated lymphocytes incubated with chloroquine (10–30 μg per culture).

Discussion

Previous studies have demonstrated that chloroquine alters some of the biochemical events associated with the *in vitro* transformation of lymphocytes elicited by PHA (13, 15). Nontoxic doses of chloroquine in the range of 10 μg/ml were shown to inhibit the incorporation of ^{14}C-thymidine and block the release of acid hydrolases from lymphocyte lysosomes. The present study demonstrates that ^{14}C-thymidine incorporation by lymphocytes stimulated with each of four different phytomitogens (PHA, PWM, Concanavalin A, and wax bean glycoprotein) is inhibited at the same dose of chloroquine (50 μg per culture, 15 μg/ml). These findings support the notion that chloroquine stabilizes lysosomal membranes, and thus prevents the release of enzymes which appear to be important in initiating the derepression of lymphocytes. Although we have confirmed and extended the observations of inhibition of ^{14}C-thymidine incorporation (13, 15), the present investigation demonstrates that chloroquine may exert an independent effect on the integrity of the cell. At lower doses of chloroquine (10–30 μg per culture) which did not completely inhibit thymidine incorporation, two types of morphologic alterations in the formation of lymphocyte lysosomes were observed, namely the development of extraordinarily large lysosomes, and the formation of degenerative foci within these structures (myelin whorls) unassociated with fusion with phagolysosomes. In some cells, the degenerative changes appeared to involve lysosomes in the initial phases of formation, as well as the larger granules. Endocytosis was observed in both transformed cells and occasionally in non-transformed cells. The extent of autophagy in these cells could not be determined because of the amorphous nature of the contents of intracellular phagolysosomes.

Several investigations (1, 9–12) have demonstrated fine structural alterations in cells treated with chloroquine, both *in vitro* and *in vivo*. These investigations have demonstrated several types of vacuoles with heterogeneous contents, including myelin figures and membranous whorls. Such changes may occur in the absence of effects on nuclear morphology. The present study has demonstrated similar morphologic effects on transforming lymphocytes. Recent

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FIG. 7. Cell from culture incubated with PHA and 10 μg chloroquine. Many vesiculated structures are seen in the Golgi zone (× 5,500).

FIG. 8. Golgi region of cell from culture incubated with PHA and 30 μg chloroquine containing numerous large vesiculated structures (× 9,100).

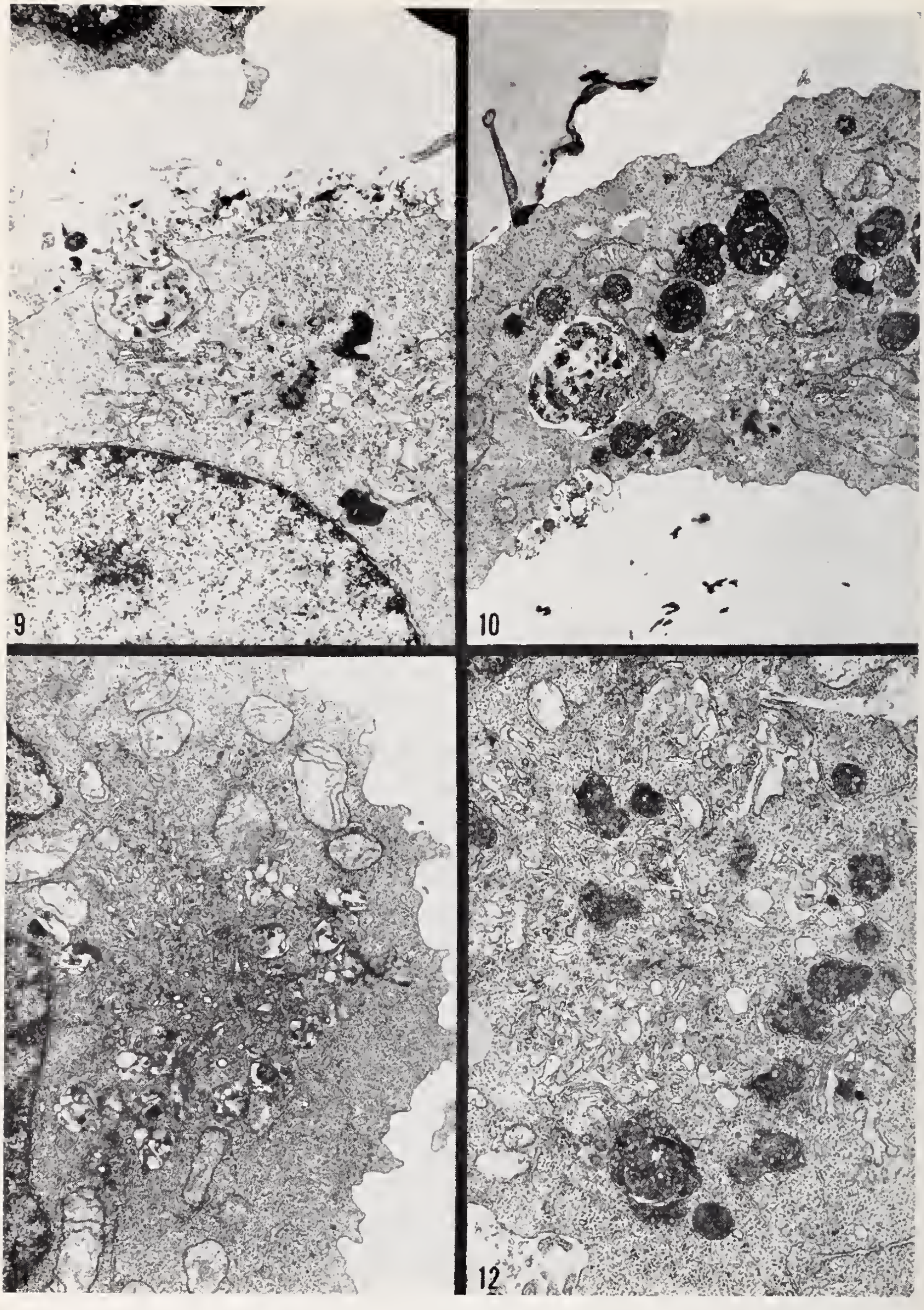

PLATE III

Cells from 72 hour lymphocyte cultures stimulated with phytomitogens in presence of chloroquine.

Fig. 9. Large vacuole containing myelin structures continuous with cell membrane and in proximity to Golgi lamellae. Culture incubated with PHA and 10 μg chloroquine ($\times$ 15,600).

studies by Fedorko, Hirsch, and Cohn (11, 12) have shown formation of toxic vacuoles in cultures of mouse macrophages and L-strain fibroblasts exposed to chloroquine. These authors have demonstrated that the effect persisted when pinocytosis was suppressed by dinitrophenol, iodoacetate, and other inhibitors (12). They suggested that the initial action of chloroquine is on the Golgi zone or smooth endoplasmic reticulum vesicles and granules, and leads to fusion of these organelles with each other and with pinosomes. In phytomitogen stimulated lymphocytes treated with chloroquine, we have demonstrated the formation of large multivesicular bodies (lymphocyte lysosomes) in the Golgi zone. However, at these doses (10–30 μg chloroquine per culture) many cells continued to undergo mitosis and contained an increased number of normal appearing lysosome-like organelles in addition to the abnormal granules. Fedorko, Hirsch, and Cohn have shown evidence that macrophages and fibroblasts exposed to chloroquine develop large vacuoles which contain some cytoplasmic organelles, indicating autophagy. The present study of lymphocyte transformation *in vitro* has failed to show evidence for autophagy in this system. Available evidence suggests that chloroquine may have a selective effect on granule formation in the Golgi apparatus of stimulated lymphocytes. The mechanism of this effect warrants further investigation.

Summary

Thymidine incorporation by human lymphocytes stimulated *in vitro* with each of four phytomitogens (phytohemagglutinin, pokeweed mitogen, Concanavalin A, and wax bean glycoprotein) was inhibited by chloroquine (50 μg per culture). At doses of 10–30 μg per culture, large multivesicular bodies and phagolysosomes with amorphous contents were observed in the stimulated lymphocytes. There was no evidence of autophagy. The findings support the concept that chloroquine may have a selective effect on the formation of lysosomes.

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FIG. 10. Large organelle containing many vesicles surrounded by several similar organelles. Culture incubated with PHA and 30 μg chloroquine (× 9,100).

FIG. 11. Vesicular bodies which appear to be forming in Golgi region. Culture incubated with PWM and 10 μg chloroquine (× 15,600).

FIG. 12. Multivesicular bodies which appear to be forming in Golgi region. Culture incubated with PHA and 10 μg chloroquine (× 16,500).

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Effect of Protein Depletion on Skin and Bone Collagen*

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Collagen is the most abundant single protein in the animal body. Although continuous synthesis and degradation are occurring, the large amount of collagen present results in half-life figures which are long compared to other proteins. Nutritional studies have usually ignored collagen, despite its abundance, because it was thought to be metabolically inert, but further analysis of the possible depot role of collagen in times of protein depletion seems warranted.

About 50 percent of the total collagen is present in bone, and about 25 percent in skin. Bone and skin collagen generally vary together in disease. Both are thinned in senile osteoporosis, Cushing's syndrome, and osteogenesis imperfecta; both are usually thickened, in generalized fashion in acromegaly, and in patchy fashion in a rare familial disorder, dermatofibrosis lenticularis disseminata, in which the x-rays show patchy areas of increased bone density (osteopoikilosis), and skin shows patchy areas of thickened dermis. Changes in the collagen content of skin and bone may not be reflected by changes in collagen concentration, since other components of these tissues may change in concentration simultaneously. A more satisfactory parameter for study of quantitative alterations in collagen in these organs is determination of the total amount present in the entire organ. Accordingly, studies of the effect of protein depletion on the total collagen content of skin and bone in mice were performed. Evidence of removal of collagen from both organs was found which resulted in qualitative changes in bone composition.

Methods

White mice were obtained from a local supplier, and from the Charles River Mouse Farms, Inc., No. Wilmington, Mass. After initial periods on the standard Purina rat chow (26% protein), groups were kept on this diet ad lib or placed on a protein depletion diet obtained from Nutritional Biochemical Corp., Cleveland, Ohio (dextrin 84%, corn oil 9%, salt mixture 4%, agar 2%, cod liver oil 1%, plus additional salts and a vitamin mixture). Water was available ad lib. After 4 days on the experimental or control diet, the mice were weighed and killed. Abdominal organs were removed and weighed individually, the entire gastrocnemius muscle was dissected free and weighed, one entire tibia was removed and cleaned of attached tissue, and a femur was similarly cleaned and a one centimeter section from the middle of the bone was obtained, carefully excluding any epiphyseal or metaphyseal cartilage. The bone samples were weighed and hydrolyzed in 6 N

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HCl at 15 lb pressure for 18 hours. The hydrolysate was analyzed for hydroxyproline by the method of Kivirikko and Leismaa (1), and for calcium by chelation with EDTA (2). The entire skin of each animal was removed from the base of the skull to the digits in each paw; underlying loose connective tissue was removed and the skin weighed, hydrolyzed, and analyzed for hydroxyproline. In some experiments, the skin was extracted at 4°C for 18 hours serially with 50 ml of 0.15 N NaCl three times, then 50 ml of 0.5 N acetic acid once. Aliquots of the salt and acid extracts and of the residue were hydrolyzed and hydroxyproline determined.

Results

Control mice gained an average of 7 percent of their body weight during the 4 days of observation; mice on the protein depletion diet lost an average of 25 percent of their weight in this period (Table I). As expected, a considerable decrease in liver and muscle weight occurred; kidney and spleen lost little weight. These data parallel those of many earlier investigators and are not reported here. The tibia showed no significant loss of weight. Skin weight was decreased an average of 25.8 percent.

BONE COMPOSITION (Table I)

The tibia showed a consistent decrease in total hydroxyproline, reflecting decreased bone collagen content which averaged 24.4 percent in the smaller mice and 15.7 percent in the larger mice. The total calcium content and weight of the bone were not changed; as a result the ratio of calcium to hydroxyproline in the tibia was increased (about 24 percent in both series of animals). To eliminate any contribution to these data by collagen of articular or growth-plate cartilage, the one centimeter section of the middle of the femur was similarly analyzed, and showed the same change in ratio of calcium to hydroxyproline.

TABLE I

Effect of Protein Depletion Diet on Bone Composition
(Data are Mean Values Plus or Minus Standard Error of the Mean)

	No. of Animals	Initial Weight (gm)	Final Weight (gm)	Tibia				Femur
				Weight (mg)	OHP* (mg)	Ca (mg)	Ca/OHP	Ca/OHP
Test	18	24.0	17.8	55.5	1.22 ± 0.09	5.75	4.78 ± 0.22	6.08† ± 0.33
Control	22	25.3	26.5	50.4	1.46 ± 0.24	5.23	3.84 ± 0.14	4.93‡ ± 0.20
p					<0.05		<0.001	<0.01
Test	10	39.3	30.0	68.3	1.14 ± 0.20	8.97	5.69 ± 1.53	8.08 ± 1.40
Control	10	39.5	42.9	73.0	1.53 ± 0.22	8.06	4.30 ± 0.85	6.24 ± 1.14
p					<0.001		<0.05	<0.01

* OHP = hydroxyproline.
† observations made on 13 animals.
‡ observations made on 16 animals.

SKIN COLLAGEN (Table II)

A decrease in the total weight of the skin, averaging 25.8 percent, was found in mice on the protein depletion diet. The hydroxyproline in the entire skin was decreased an average of 16.9 percent, reflecting a decrease in the total amount of collagen in the skin. Comparable decreases were found in the amount of hydroxyproline present on the salt-soluble and acid-soluble collagen fractions (19.1 percent and 34.3 percent, respectively); somewhat less of a decrease was found in the insoluble collagen fraction (13.7 percent), although the greatest absolute fall in hydroxyproline occurred in that fraction.

EFFECT OF CALORIC RESTRICTION

The animals on the protein depletion diet consumed an average of 7.99 gm of the diet per day, whereas mice on the control diet ingested an average of 8.98 gm/day. These diets were approximately equivalent in caloric value. In one experiment, mice on the control diet were restricted to the average food intake of mice on the protein depletion diet. As is shown in

TABLE II

Effect of Protein Depletion Diet on Skin Collagen
(Data are Mean Values Plus or Minus Standard Error of the Mean)

Total Weight and Hydroxyproline

	No. of Animals	Total Skin Weight (gm)	Total Skin Hydroxyproline (mg)
Protein depletion diet	10	3.661 ± 0.157	54.2 ± 2.4
Control diet	10	4.934 ± 0.389	65.2 ± 3.8
p		<0.01	<0.001

Collagen Fractions

	No. of Animals	NaCl Extractable Hydroxyproline (μg)	Acid Extractable Hydroxyproline (μg)	Insoluble Hydroxyproline (mg)
Protein depletion diet	14	165 ± 3.6	40.9 ± 2.8	3.47 ± 0.39
Control diet	9	204 ± 3.4	62.3 ± 2.4	4.02 ± 0.49
p		<0.001	<0.001	<0.001

TABLE III

Comparison of Effect of Protein Depletion Diet and of Comparable Amounts of Standard Diet

	No. of Animals	Initial Weight (gm)	Final Weight (gm)	Tibia	
				Weight (mg)	OHP (mg)
Protein depletion diet	10	41.3	29.8	65.1	0.859 ± 0.031
Restricted usual diet	10	41.1	35.6	80.6	1.04 ± 0.06
p					<0.001

Table III, on this restricted caloric intake the mice lost weight, although not as much as those on the protein depletion diet; total tibial hydroxyproline was decreased in both groups of mice, but much more markedly in those receiving the protein depletion diet.

Discussion

These observations show that collagen, measured as hydroxyproline, serves as protein reservoir which is mobilized when mice are put on a diet devoid of protein. Since most of the collagen is in the bone and skin, these two sites show evidence of removal of this protein. In the skin, the recently synthesized, soluble collagen showed a greater percentage loss, but the larger insoluble fraction showed a greater decrease in absolute hydroxyproline content. Preliminary data obtained in additional studies show evidence for increased collagenase activity in the skin of the mice on the protein depletion diet, using the method of Eisen, Jeffrey, and Gross (3). The liver and muscle of these animals showed earlier and proportionately more marked changes in weight and protein content, but the absolute amounts of collagen mobilized in 4 days in the mice in these experiments was quite considerable, even though the percentage loss was not great. The concentration of collagen in the wet, fresh skin actually increased, since water, fat, glycogen, and other proteins were also changing, but the total collagen in the entire skin and tibia was decreased significantly.

Bone collagen removal resulted in a surprising qualitative alteration in bone composition. Apparently, removal of mineral and matrix collagen did not occur at the same rate in these experiments. The mineral phase showed no alteration in the period of study, resulting in a change in the calcium to hydroxyproline ratio in the tibia and in a segment of the femur. A similar rise in calcium to hydroxyproline ratio was reported in undernourished cockerels (femur) and pigs (humerus) (4). Hydrocortisone caused a similar qualitative alteration in the bone of chicks, especially when administered in large enough doses to influence body weight (5). The opposite change, mobilization of mineral without removal of collagen, has been reported in lactation (6). These observations show that influences on bone mineral and matrix removal must be analyzed separately, and that statements in the literature that bone matrix and mineral are always removed concomitantly should be questioned.

It cannot be stated that the animals in this study developed osteoporosis, since total bone mass did not decrease. Starvation is known to produce osteoporosis in humans (7). Prolonged studies in animals on protein or calorie restriction show markedly decreased bone mass, correctable by nutritional rehabilitation, but these data are difficult to interpret as osteoporosis since they seem to be failure of growth rather than increased removal of bone. Further studies with adult animals will be necessary to detect whether more prolonged underfeeding can result in removal of mineral as well, decreasing total bone mass. The observations reported here do show that collagen can be removed from bone and skin during relatively short periods of nutritional deficiency. No comparable data exist in humans, but increased collagen breakdown is thought to be the basis for the increased urinary excretion of peptide-bound hydroxyproline which occurs in children with Kwashiorkor (8), and when obese patients are fasted (9).

Summary

Mice on a protein depletion diet for 4 days showed significant decreases in total collagen, measured as hydroxyproline, in skin and bone (tibia). The salt and acid-soluble as well as the insoluble collagen fractions of the skin showed decreases. Calcium content of the bone was not altered, and calcium to hydroxyproline ratios were increased in the tibia and a section from the femur.

These data suggest that collagen can serve as a reservoir protein which is mobilized during periods of protein depletion, and that bone collagen can decrease without concomitant removal of mineral.

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Urinary Kynurenine Excretion in Rheumatoid Arthritis following an Overnight Fast*

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It has been repeatedly demonstrated by various investigators that patients with rheumatoid arthritis excrete increased quantities of tryptophan metabolites of the kynurenine pathway in their urine, chiefly kynurenine (1–3). The reason for the increased excretion is not known. Postulated mechanisms have included pyridoxine deficiency (4), increased hepatic tryptophan pyrrolase activity (2), and altered renal handling of kynurenine (5). In the studies that have been done, 24 hour urine output of kynurenine was measured while on self-selected diets (2), or following administration of a tryptophan load (4). Both types of study are open to criticism. It may be argued that patients on self-selected diets may differ in their tryptophan intake or gastrointestinal absorption. The loading experiments may be criticized on the basis that tryptophan itself is a potent inducer of tryptophan pyrrolase, and the amount of tryptophan used may impose an entirely unphysiological stress upon the metabolic machinery. The average daily intake of tryptophan is one gram, and in the loading experiments a minimum of two grams of 1-tryptophan are used.

In order to circumvent these objections, a group of 14 patients with rheumatoid arthritis and a control group of 14 patients were studied under fasting conditions in order to determine whether the increased excretion of kynurenine is manifest even under "basal" conditions.

Materials and Methods

Patients were from the Arthritis Clinic of The Mount Sinai Hospital. They had either a definite or classic rheumatoid arthritis as defined by the American Rheumatism Association Criteria (6). Control patients were healthy laboratory personnel and patients with fibrositis syndrome. All patients were instructed to eat nothing past 10 PM, and upon awakening to empty their bladder and discard the urine. Food and medication were withheld until the end of the experiment. They were given 500 cc of water to drink. After 2½ hours, the total urine output was collected in a bottle containing 1 cc of concentrated hydrochloric acid. Kynurenine determinations were performed by the method of Tompsett (7).

A group of four rheumatoid patients were instructed to fast and drink 250 cc of water every hour. Urines were collected at hourly intervals for 4 hours. The patients were then fed a meal containing 400 mg of tryptophan, and urine collected at hourly intervals for 6 more hours.

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TABLE I

Two and One-Half Hour Excretion of Kynurenine after an Overnight Fast (mg)

Rheumatoid Arthritis	Control
1. 0.75	1. 0.53
2. 0.80	2. 0.18
3. 0.88	3. 0.16
4. 0.25	4. 0.22
5. 0.99	5. 0.14
6. 0.08	6. 0.19
7. 0.39	7. 0.06
8. 0.50	8. 0.12
9. 0.30	9. 0.27
10. 0.10	10. 0.21
11. 0.48	11. 0.16
12. 0.34	12. 0.20
13. 3.73*	13. 0.24
14. 0.10	14. 0.22
Mean: 0.458* (±SD) (0.310)	Mean: 0.207 (±SD) (0.107)

* mean calculated without the value of 3.73 mg.

Results

In all the four patients studied, the increased kynurenine excretion following a meal returned to preprandial levels within 4 hours. This demonstrated that an overnight fast is sufficient to eliminate immediate dietary factors.

The control group of 14 patients excreted a mean of 0.207 ± 0.107 mg kynurenine per 2½ hours. Among the patients with rheumatoid arthritis, the mean excretion was 0.458 ± 0.310 mg per 2½ hours. The difference between the two groups is statistically significant ($p < 0.02$). Seven of the 14 patients with rheumatoid arthritis excreted more than 2 SD above the mean of the control group. These results are shown in Table I.

Discussion

In this study, one-half of the patients with rheumatoid arthritis excreted increased quantities of kynurenine even under basal conditions. This would seem to indicate that the abnormality responsible for the increased excretion of tryptophan metabolites is independent of tryptophan intake. This obviates the need for 24 hour urine collections or tryptophan loading in future studies, thereby eliminating the objection inherent in loading experiments and self-selected diet studies.

The significance of the increased excretion of tryptophan metabolites is not known. The small number of patients in the present study prevented any meaningful analysis of clinical correlates of kynurenine excretion among the patients with rheumatoid arthritis; however, tissue wasting and activity of the disease did not seem to correlate with urinary excretion.

Summary

Seven of 14 patients with rheumatoid arthritis excreted significantly increased quantities of kynurenine after an overnight fast. In a group of four rheumatoid patients the increased excretion of kynurenine following a meal containing 400 mg of tryptophan returned to normal within 4 hours.

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Antigenicity of Connective Tissue Components*

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Until recently, a view prevailed that connective tissue components, particularly polysaccharides and collagen, were either non-antigenic or poorly so. Several papers indicated the apparent inability to produce antibodies in rabbits immunized with hyaluronate isolated from connective tissues or bacteria (1–5). A report of antibodies to chondroitin sulfate later was not confirmed by Boake and Muir (6) using highly purified polysaccharide. Finally, the apparent lack of antigenicity of collagen was observed by many workers (7).

More recent studies indicate that these earlier views must be modified: Many connective tissue components are antigenic. Probably the major reason for this change is that compounds of protein and polysaccharide isolated from connective tissues by mild methods have been used as antigens. Formerly, attention was directed primarily toward defining the chemical structure of the polysaccharides; proteolytic enzymes or strong reagents, particularly alkali, were used during isolation to remove as much protein as possible from the polysaccharides. Of course, this view suggests that the protein itself, or the linkage region between protein and polysaccharide, is the chemical site responsible for antigenicity; this view is supported by the evidence described in the paper. We will chiefly discuss the antigenic properties of connective tissue proteinpolysaccharides, for this has been the area of our own interest. A brief description of the antigenicity of basement membranes and collagen will also be included (Table I).

Cartilage Proteinpolysaccharide

The chondroitin sulfate in the matrix of cartilage is covalently bound to non-collagenous protein, forming macromolecules designated proteinpolysaccharides (8). Proteinpolysaccharides have been shown to be antigenic in rabbits and guinea pigs (9–11). Antibodies to the cartilage proteinpolysaccharides have been demonstrated by a number of different techniques, including: (1) classical quantitative precipitation; (2) double diffusion studies in agar; (3) tanned cell agglutination; and (4) immunofluorescent studies using sections of cartilage. The immunized animals (rabbits and guinea pigs) also give positive skin tests with the proteinpolysaccharides.

Cartilage proteinpolysaccharides from 11 different species cross-react (12). Inhibition studies using tanned sheep red blood cells indicate that the cartilage proteinpolysaccharides from these 11 species share at least one *common*

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TABLE I

Antigenic Determinants of Some Components of Connective Tissues

Cartilage Proteinpolysaccharide	Synovial Fluid Hyaluronate	Basement Membrane	Collagen
A minimum of two antigenic determinants are present; one is common to many cartilage proteinpolysaccharides, and the other is species specific.	A minimum of two antigenic determinants are present; one is very similar to the common determinant in cartilage proteinpolysaccharide. In addition, hyaluronate isolated from pathological synovial fluid contains an antigenic component immunologically identical to a serum interalpha trypsin inhibitor.	Basement membranes probably contain many antigenic determinants. Basement membranes of epithelial and mesenchymal origin cross-react, but each also contains specific determinants.	Collagen probably contains many antigenic determinants-some are species specific, and some cross-react widely. The cross-reacting determinants are relatively inaccessible in the native configuration of collagen.

antigenic determinant. Another antigenic determinant which is *species specific* has been demonstrated in proteinpolysaccharides from human, cow, and pig cartilages (9). It is not clear whether the common and species specific components of the proteinpolysaccharide are both present on the same proteinpolysaccharide or whether they are actually present on two different molecules. The reactive components can be separated by hyaluronidase digestion of the proteinpolysaccharide; immunoelectrophoresis then shows that the common component migrates more rapidly than the species specific component.

After digestion of the cartilage proteinpolysaccharides with trypsin and chymotrypsin, and fractionation on columns of Dowex 1 resin, products can be isolated that contain two chondroitin sulfate chains joined by just a few amino acids (13). These products have been termed doublets. Doublets from cow, lamprey, stingray, and sturgeon cartilage proteinpolysaccharides contain two residues of serine, and one residue or more of glycine and glutamic acid, suggesting that the doublet may be a structural unit found in many types of cartilage. Inhibition studies suggest that the antigenic determinant shared by cartilage proteinpolysaccharides of 11 species is located in the doublet. Digestion with hyaluronidase does not decrease the immunological reactivity of the doublet, indicating that the major part of the chondroitin sulfate chain is not essential. The common determinant is probably closely associated with the region where chondroitin sulfate is linked glycosidically at its reducing end via two residues of galactose and one residue of xylose to the hydroxyl group of a serine residue in the protein core (14) (Table II).

Indirect immunofluorescent techniques have been used to localize the proteinpolysaccharides in tissues (15, 16). When cartilage sections were studied with antiserum to cartilage proteinpolysaccharides, the fluorescence was primarily in the lacuna of the chondrocyte and perilacunar area. If the cartilage sections were pretreated with testicular hyaluronidase, the fluorescence was diffusely distributed throughout the matrix. Normal synovial lining cells also show fluorescence when studied with this antiserum (17, 18). When sections of

TABLE II

Chemical Composition of Some Components of Connective Tissues

Cartilage Proteinpolysaccharide	Synovial Fluid Hyaluronate	Basement Membrane	Collagen
Chondroitin sulfate chains (galactsm.-SO_4-glucuronate, mol wt 50×10^3) are linked via galactose and xylose to β-OH of serine in the protein. Other polysaccharide chains are keratan sulfate (galactose-SO_4-glcsm.).	Polysaccharide chain (glcsm. and glucuronate, mol wt 10^6) is linked via galactose to β-OH of serine and threonine in the protein*.	From renal glomerular basement membrane or lens capsule of eye, collagen-like protein is isolated containing two carbohydrates; one is a disaccharide (glucose and galactose) linked to δ-OH of hydroxylysine; the other is a heteropolysaccharide (galactose, mannose, hxsm, sialate, and fucose) probably linked to asparagine.	Neutral salt extraction of tendon, dermis, or cartilage yields a soluble collagen called tropocollagen, containing three polypeptides (α-1, α-1, and α-2) wound in a helix.

* other links may exist.

other tissues have been studied with antiserum to cartilage proteinpolysaccharide, fluorescence has been found in: (1) basement membrane of glomerulus; (2) basement membrane of renal tubule; (3) sarcolemma of cardiac and skeletal muscle; (4) basement membrane of tracheal mucosa and thyroid; and (5) endothelium and basement membrane of blood vessels.

Using antiserum prepared to cartilage proteinpolysaccharide a new component has been isolated from human synovial fluid, which on double diffusion gives a reaction of complete identity with the *species specific* component present in human cartilage proteinpolysaccharide (19). This component has a molecular weight of approximately 130,000 and is predominantly a protein; a small amount of hexosamine is present (20). The amount of new component in the joint fluid is increased in acute synovitis, but is decreased, apparently specifically, in chronic rheumatoid arthritis. Using the indirect immunofluorescent technique with specific antiserum, new component was shown to be present in normal synovial membrane lining cells but was practically absent in rheumatoid synovial cells. Similar findings were observed in normal and rheumatoid synovial cells grown in tissue culture (17). New component was present in the media obtained from cultures of normal synovial cells, indicating that some of the new component in synovial fluid is produced by the synovial lining cells; the cartilage itself may be an additional source of new component.

Hyaluronate

It is not entirely certain that hyaluronate in connective tissues is bound to protein. One thing does appear clear: If protein is covalently bound to hyaluronate, the amount of protein is so small that it has sometimes been dismissed as a "contaminant." In some reports (21, 22) hyaluronate has been considered to be essentially free of protein, although chemical analysis revealed 2 percent. In another report (23), a molar ratio of nitrogen to hexosamine of 1.00 was said to be evidence for the absence of protein. However, chemical analysis alone is not sufficient to exclude the presence of traces of bound protein. There are now five reports that indicate the firm linkage of hyaluronate to protein: The sources of the hyaluronate are cow vitreous (24), umbilical cord (25), rooster comb (26), and human synovial fluids—normal and pathological (27–29).

From normal synovial fluid a compound of hyaluronate containing 2% protein has been isolated and termed hyaluronateprotein (27). When the protein was labeled with I-131 and the compound subjected to zone electrophoresis in buffers ranging in pH from 4.5–10.6, or in high salt concentration, or in 6 M urea, the polysaccharide (measured by hexuronic acid) and protein (determined by I-131 counts) were always found to migrate together. From animals immunized with hyaluronate in Freund's adjuvant, specific antiserum can be obtained which reacts with hyaluronate in the following systems: (1) Antiserum inhibits the electrophoretic migration of hyaluronate labeled with I-131 (30); (2) In immunofluorescent tests, antiserum localizes to the lining cells and their intercellular matrix in the synovial membrane (31). The presence of hyaluronate in the matrix was previously suggested by metachromatic staining abolished by streptococcal hyaluronidase (32). The production of hyaluronate by synovial cells was subsequently confirmed by electron microscopic radioautographic studies (33); and (3) In double diffusion studies, antiserum to hyaluronate forms a precipitin line with hyaluronate (18).

Antiserum to hyaluronate cross-reacts with proteinpolysaccharides of cartilage (Table I). This can be shown by agar double diffusion and tanned cell agglutination studies (18, 30). Antiserum to cartilage proteinpolysaccharide forms one precipitin line on double diffusion which gives a reaction of complete fusion with the *common* antigenic component of cartilage proteinpolysaccharide. Hyaluronate has chemical similarities with cartilage proteinpolysaccharides (i.e., the presence of galactose, galactosamine, and glycosidically linked serine) which may account for its cross-reaction with cartilage proteinpolysaccharide (Table II). It has been suggested that hyaluronate may be contaminated with cartilage proteinpolysaccharide; evidence against this is the absence of xylose, the presence only of common component, and the presence of glycosidically linked threonine. Thus, part of the configuration of hyaluronate and cartilage proteinpolysaccharide must be similar, but only with the common site of the latter. Hyaluronate appears to have at least one antigenic determinant not shared with cartilage proteinpolysaccharide. Absorption of antiserum to hyaluronate with cartilage proteinpolysaccharide did not affect the results in either the electrophoretic or immunofluorescent systems described above.

Antiserum to hyaluronate isolated from human synovial fluid also cross-reacts with hyaluronate produced by Group A streptococcus (18). It is rather remarkable that despite the low protein content of the streptococcal hyaluronate (<0.3 percent), it contains chiefly two amino acids (serine and threonine) and a neutral sugar (galactose), components present in human hyaluronate. Perhaps these amino acids and the sugar are involved in the cross-reacting antigenic determinants. Stoolmiller and Dorfman (34) also found traces of protein (0.1–0.2 percent), galactose, and galactosamine in streptococcal hyaluronate. However, it is not yet known whether streptococcal hyaluronate is covalently linked to peptides, and it is possible that contaminants present in the isolated streptococcal hyaluronate account for both the chemical findings and the cross-reactivity with human hyaluronate and cartilage proteinpolysaccharide. In fact, there are two observations which raise doubts about the linkage of peptides to streptococcal hyaluronate: (1) Chloramphenicol and puromycin fail to inhibit synthesis of streptococcal hyaluronate; and (2) Reduction of streptococcal hyaluronate with NaB^3H_4 reveals glucosamine at the reducing terminus and no evidence for covalent binding to hydroxyamino acids (35). In a mammalian cell strain with a temporary life span in tissue culture (human synovial membrane cells), inhibition of protein synthesis partially inhibits the synthesis of hyaluronate; in established (i.e., permanent) mammalian cell lines (rat eye or mouse embryo fibroblast), almost complete cessation of hyaluronate synthesis occurs when protein synthesis is inhibited (36). Whether this difference in mammalian cells is related to establishment in cell culture is not known. Obviously, there is much to be learned about the possible linkage of streptococcal hyaluronate to protein, and the relation of human and streptococcal hyaluronate synthesis to formation of the presumed protein moiety. If covalently linked, the intriguing possibility exists that hyaluronate is attached to a peptide whose synthesis is insensitive to the protein-synthesis inhibitors used. It should be pointed out that in the case of cartilage proteinpolysaccharide, where the linkage of protein to polysaccharide is well established, and where protein constitutes a greater proportion of the whole compound, inhibition of protein synthesis brings polysaccharide formation to a halt (37, 38).

There is evidence that antiserum to hyaluronate is reactive with the protein moiety or with sites of linkage between polysaccharide and protein. The hyaluronate can be digested with: (1) testicular or streptococcal hyaluronidase to produce polysaccharide fragments, and there remains an intact protein bound to a small residue of sugars (30); or (2) with papain or trypsin, which appear to hydrolyze the protein but leave the polysaccharide chains intact (27). Antiserum absorbed with hyaluronate digested with proteolytic enzymes is no longer reactive in immunofluorescent tests with the synovial membrane, but antisera absorbed with hyaluronidase-digested hyaluronate remains reactive (31).

Hyaluronate from pathological synovial fluids contains 7 to 10 percent firmly bound protein (28). Antiserum prepared to pathological hyaluronate reacts with one *serum* protein which has been identified as the inter-alpha trypsin inhibitor (39) (Table I). In diseases of the joint, inter-alpha trypsin inhibitor from the serum enters the articular cavity and becomes bound to the hyaluronate, forming a macromolecule with certain physical properties different from normal hyaluronate (28).

Basement Membranes

The chemical composition and antigenic properties of basement membranes have received a great deal of attention in the past few years. However, the precise definition of basement membranes and their morphologic features have not been clarified and are undergoing continual change and modification. It might be said that basement membrane is a relatively thin layer (containing amorphous material and thin filaments called reticulin) interposed between epithelium and underlying connective tissue (lamina propria) and mesenchymal cells (40). Because of its completely extracellular location, it has been suggested by those concerned with fine structure that the term basal lamina be used, particularly when referring to the substratum of epithelium; however, basement membrane is the term more widely used by immunologists and chemists. Note that fibers with periodicity characteristic of collagen have not been included in the definition of basement membrane, but striated fibers have been described in certain locations in basement membranes or connective tissues within mammalian brain, eye, skin, and peripheral nerves, or in insects (41). There are yet other fibers thought to be distinct morphologically from collagen or reticulin, and which have been called "anchoring fibrils" (42, 43). These fibrils run from the collagen in the lamina propria through the basement membrane and appear to attach to the epithelial cell surface; they may play a role in maintaining the firm attachment between epithelium and connective tissue. Fibers in the basement membrane have usually been described as reticulin, which connotes many properties similar to collagen, such as digestibility by collagenase, resistance to trypsin, and similar amino acid profiles. There are also differences, based on morphological, and especially tinctorial properties: Reticulin is argyrophilic, and its associated interfibrillar material stains deeply with the PAS reaction (44).

Most of our information on the chemical composition of basement membranes comes from preparations of the renal glomerulus; anterior lens capsule has also been studied (45, 46). There is chemical similarity of the collagen of basement membranes (reticulin) and tendon collagen, but there are also interesting differences (Table II): There is, in the former, more carbohydrate, especially glucose, more hydroxylysine and hydroxyproline, less glycine and lysine, and the presence of amino acids deficient or lacking in tendon collagen, such as half-cystine, tryptophan, histidine, methionine, and tyrosine. A characteristic feature of basement membrane collagen and of skin collagen is the glucosylgalactose linked glycosidically to δ-OH of hydroxylysine (47, 48). Another component in basement membranes is a glycoprotein, containing galactose-mannose-hexosamine-sialate-fucose, possibly linked to asparagine of the protein; a similar glycoprotein has been noted in fetuitin, thyroglobulin, and acid α-1 glycoprotein of serum. Note that basement membrane contains none of the anionic polysaccharides characteristic of ground substance of connective tissue. It seems possible then, that those carbohydrates associated with reticulin not only modify its fiber forming characteristics, but also give it its characteristic PAS and argyrophylic reactions (44), and may play a role in limiting the aggregation of the collagen molecules which is responsible for the characteristic banding of tendon or skin collagens (46).

The immunologic properties of basement membranes have been intensively investigated. There are several reasons for this widespread interest: (1) Basement membranes have been divided into those which are primarily epithelial (such as lens capsule) or primarily mesenchymal (such as splenic tissue), and it has been of interest to compare their antigenic cross-reactions (49); (2) The whole subject of basement membrane antigenicity is tied up with nephrotoxic nephritis in animals, and with comparable human diseases, especially glomerulonephritis and Goodpasture's syndrome (pulmonary disease and glomerulonephritis) (50, 51); and (3) The basement membrane in the renal glomerulus is the site where immune complexes may be deposited in serum sickness or systemic lupus erythematosus, diseases in which nephritis may also occur (52).

This paper is not the place to review the subject of nephrotoxic nephritis, which defines immunologic injury to the kidney on the basis of the interaction *in situ* of renal glomerular basement membrane antigens and their antibodies (52). Briefly, in classical nephrotoxic nephritis, renal antigens from animal A are used to immunize animal B; antiserum from B injected into A becomes fixed to renal glomerular basement membrane. It appears that this step may or may not cause nephritis, but the next event always does: the formation by animal A of immunoglobulins to the γ globulins fixed on the basement membrane. Preparations of renal glomerular basement membranes themselves may be used to immunize animals; the antibodies formed fix to the glomerulus and usually (but not always) (53) cause nephritis. The immunoglobulins, detected by a fluorescein-labeled antiserum to γ globulin, are revealed by the smooth, linear staining along the endothelial side of the basement membrane of the glomerulus.

Renal glomerular basement membrane contains components that cross-react with purified antisera to epithelial basement membrane (lens capsule), as well as with antiserum to mesenchymal basement membrane (spleen); absorption of the antiserum to lens with spleen removes almost all the reactivity with kidney, but does not abolish reactivity with lens. Absorption of anti-spleen or anti-kidney sera with lens removes their reactivity with lens, and while the latter antiserum still localizes in the kidney, its nephrotoxicity is apparently eliminated (49). It is interesting that anti-lens serum, which is known to cross-react with kidney, still fails to produce nephritis; whether this is due to the low titre of antibody, or to other reasons, is not known. Antisera to other organs are known to cross-react with renal basement membrane antigens. Anti-lung serum, for example, produces nephritis (54). Indeed, the cross-reactivity of antigens in basement membranes of lung and renal glomerulus has been clearly shown and is of great relevance to the etiology of Goodpasture's syndrome (51).

Thus, similarities and differences exist between basement membrane antigens primarily of epithelial origin (lens capsule), and primarily mesenchymal in origin (renal glomerulus, and alveolar septa). The chemical basis for antigenic properties is not entirely known. Purified basement membranes from lens, glomerulus, and alveolar septa form two precipitin lines against their antisera, and these lines appear to fuse in a reaction of identity (55).

One antigenic component appears to be of relatively high molecular weight and is composed of the collagen-like protein with both the heteropolysaccharide and disaccharide units attached (56). The other antigen is thought to be a glycoprotein which lacks glucose and has an amino acid composition unlike that of collagen (55). The nature of this component remains to be determined.

Collagen

Collagen is the most abundant protein in mammals, the dominant protein of dermis, bone, cartilage, and tendon, and in its most common form is insoluble in water. It occurs in tissues as a fibrous material that is entirely extracellular, and that is one of the principal structural elements of the animal body (44, 57).

The subject of collagen's antigenic properties is a complex one because of the use of preparations which differ in physical properties and chemical composition obtained in a variety of ways from many different species. It is clear from the outset that insoluble material is not likely to be suitable for either chemical or immunological studies, and it is not surprising that those working with such preparations usually reported them to be non-antigenic. However, Paz and her co-workers (58) described weak complement fixation reactions using sera of rabbits immunized with insoluble collagen from chickens. In general, most studies have been carried out using preparations of soluble collagen extracted in neutral salt or dilute acid solutions. The word tropocollagen has been applied to the salt-soluble collagen, but both this and the acid-soluble collagen have the same amino acid compositions, the same molecular weights (about 320,000), the same molecular dimensions (3,000 × 15 Å), and the same sedimentation coefficients (3.1 S) (44). The tropocollagen molecule is composed of three separate primary protein chains, each helically coiled to form a super-helix, and all three chains (called α-1, α-1, and α-2 are held together by hydrogen and by covalent bonds. Attached to the N-terminal portion of the collagen polypeptide is a "non-collagenous" part of the chain called the telopeptide: No hydroxyproline is present, and tyrosine, aspartic, and glutamic acids are present in high concentrations (57). Trypsin or pronase digestion releases these telopeptides without affecting the tropocollagen. Tropocollagen is antigenic in a wide variety of animal species (59). Collagenase digestion of tropocollagen destroys the reactivity between the antigen and antiserum. The telopeptides are responsible for some of the immunogenic properties of the whole molecule, but whether these or the triple helical configuration itself is responsible for species specificity is not entirely clear. Species specificity as well as cross-reactivity have been demonstrated by precipitin, immunofluorescent, tanned cell, and complement fixation tests (7, 59–62).

Warming tropocollagen at pH 4 partially separates the triple helix into mixtures of single or linked chains. Such preparations (parent gelatin) retain immunogenic properties. They show reduced species specific reactions compared to native molecules when delayed hypersensitivity is sought (63).

Further heating of parent gelatin at high temperatures causes progressive irreversible degradation to the less sharply defined material called gelatin. Such products retain weak antigenicity, which can be enhanced by adding tyrosyl residues (7, 64). Delayed skin reactions occur with widespread cross-reactivity, but there is only minimal preservation of species specificity, as judged by skin tests. Kirrane and Glynn (59) conclude that the "narrow species specificity of the response to native collagen implies that the immunological determinants of gelatin are inaccessible in the native configuration of the molecule (i.e., the triple helix), and distinct from those responsible for the specificity of the native protein."

Studies with peptides isolated from collagenase-digested soluble collagen suggest that those peptides containing polar groups are more potent antigens than soluble collagen (65). Synthetic peptides containing particularly glycine and proline are immunologically active; while they appear unable to inhibit preciptin reactions between soluble collagen and its antiserum, they do form precipitin reactions themselves with anti-collagen serum (66).

Concluding Comments

In the past few years there has been widespread interest in the antigenic properties of connective tissue components. This has come about chiefly because these components, particularly proteinpolysaccharides, have been isolated by methods designed to avoid extensive degradation and preserve antigenicity, which appears to reside in the protein moiety or in the area of the link between protein and carbohydrate. Great advances have been made in elucidating the chemical composition of proteinpolysaccharides and collagens from basement membrane and from skin. A great deal is now known about the species specific reactions of all these components, and about reactions which are more widespread and common to many species. The time is approaching when it will be possible to relate all these specific and shared immunological reactions with the chemical composition and spatial configuration of parts of the molecules.

There remains for consideration possible relations between these immunological reactions and connective tissue diseases. An immune response to normal or altered connective tissue components constitutes a basis for autoimmune theories in the pathogenesis of connective tissue diseases (67–69). More recently, and largely stemming from the work of Kaplan (70), attention has been focused on a type of immune response that can be called cross-immunity: Human infection with an exogenous agent, especially the Group A streptococcus, may lead to the formation of antibodies with possible widespread cross-reactivity to connective tissues (cardiac and synovial tissues, glomerulus, and vascular basement membrane), and result in diseases such as acute rheumatic fever or glomerulonephritis (44, 71). It is now apparent that connective tissue components, such as proteinpolysaccharides, and collagens from basement membranes and skin, which may differ so widely in physical properties, may also have regions of similar or identical chemical composition, and so share antigenic determinants. Indeed, immunofluorescent studies reveal that antiserum to cartilage proteinpolysaccharides may show widespread localization in other connective tissues; a major reacting component may be basement membranes (18, 72). Immunological cross-reactions involving basement membranes selectively, or diffusely at various locations (lung, eye, vascular system, and kidney), could set into motion the complex inflammatory processes which are associated with the connective tissue diseases.

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Bence Jones Protein: A Molecular Entity for Studies on the Structural and Genetic Control of Antibody Specificity*

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> On the 1st of November 1845 I received from Dr. Watson the following note, with a test tube containing a thick, yellow, semi-solid substance:—"The tube contains urine of very high specific gravity; when boiled it becomes highly opake; on the addition of nitric acid it effervesces, assumes a reddish hue, becomes quite clear, but, as it cools, assumes the consistence and appearance which you see: heat reliquifies it. What is it?" . . . *Henry Bence Jones* (1).

Over 25 years ago, Dr. Alexander B. Gutman (2) directed his attention toward elucidation of the plasma protein alterations produced by disease; in particular, his studies distinguished the hypergammaglobulinemia associated with chronic infectious and inflammatory diseases from that associated with multiple myeloma. By application of combined electrophoretic, ultracentrifugal, and immunochemical techniques, Gutman and associates (3, 4) were able to distinguish myeloma proteins from Bence Jones proteins, and subsequently demonstrated unequivocally the presence of Bence Jones proteins in the sera of certain patients with multiple myeloma. Gutman's interpretation of plasma protein electrophoretic profiles established the importance and contributed to the universal use of electrophoretic analyses as a diagnostic tool in clinical medicine.

The significance of the unusual urinary protein, first recognized over 100 years ago by Bence Jones (1) and MacIntyre (5), has become apparent only in the last decade. The revelation that the Bence Jones protein represents the light polypeptide chain common to all classes of immunoglobulins (6, 7) has established this readily available material as an invaluable source of homogeneous protein for studies designed to elucidate immunoglobulin structure. Bence Jones proteins occur either as monomers (mol wt 22,000), or as disulfide-linked dimers (mol wt 44,000), or both, i.e., a monomer-dimer mixture (8). Two distinct types of Bence Jones proteins and light chains, termed κ and λ[1], have been distinguished immunochemically (9, 10). The κ and λ pro-

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[1] The designation of κ and λ applies to isolated light chains and Bence Jones proteins. The light chains present on intact immunoglobulin molecules are referred to as type K or type L (Bull WHO 30:447, 1964).

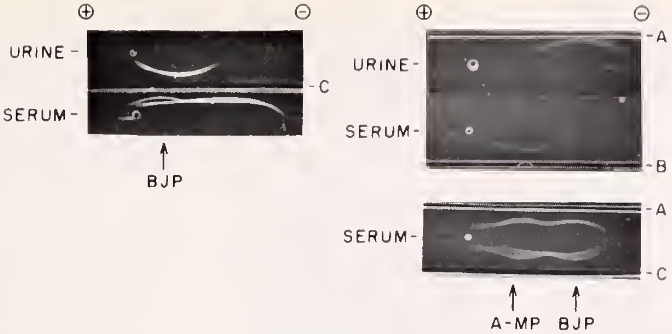

Fig. 1. Immunochemical detection of Bence Jones proteinemia. Immunoelectrophoresis of serum and urine from two patients with multiple myeloma. The antisera utilized were: anti-κ Bence Jones protein, antiserum A; anti-IgA, monospecific antiserum B; anti-IgG (Cohn Fr II), antiserum C. LEFT (patient one): The precipitin arcs formed by reaction of antiserum C with the serum and urinary Bence Jones proteins (BJP) are indicated by the arrow. RIGHT (patient two): The precipitin arcs formed by the serum and urinary Bence Jones proteins (κ type) and serum A myeloma protein (A-MP) are indicated by arrows.

teins differ widely in primary structure as evidenced by comparative analyses of tryptic peptide maps and amino acid sequences (11). Studies of the molecular differences among Bence Jones proteins have provided a structural basis for antibody specificity.

Bence Jones (1) suggested a possible association between the occurrence of the urinary protein that bears his name and the disease "mollities ossium," i.e., multiple myeloma. Indeed, the recognition of Bence Jones proteinuria has proven useful for diagnostic purposes, and variations in the urinary concentration of the protein have provided an index of response to therapy (12). The use of immunochemical techniques, particularly immunoelectrophoresis, has facilitated the detection of Bence Jones proteins. Bence Jones proteinuria occurs in at least 60 percent of patients with multiple myeloma, and in many cases Bence Jones proteins are readily detected in sera (Fig. 1). Bence Jones proteins were isolated from both the serum and urine of each of ten patients, and the identity of the homologous serum and urinary Bence Jones protein was established by comparison of antigenic determinants, sedimentation behavior, electrophoretic mobility, and thermal solubility properties (13). A variety of pathophysiological manifestations of renal disease have been described in association with Bence Jones proteinuria (13), and all ten patients with Bence Jones proteinemia had impaired renal function. The accumulation of Bence Jones proteins in serum is dependent upon the rate of synthesis, catabolism, and excretion of the protein (14). Metabolic studies utilizing isotopically labeled Bence Jones proteins (14, 15) revealed that both catabolism and urinary excretion contribute to the removal of Bence Jones proteins. Thus, the amount of Bence Jones proteins in the urine represents only a portion of the total Bence Jones proteins synthesized. Further, the rate of catabolism of Bence Jones proteins is dependent upon the renal functional capacity, i.e., catabolism is considerably reduced by impaired renal function. The specific role of the kidney in the endogenous catabolism of Bence Jones proteins is evident from

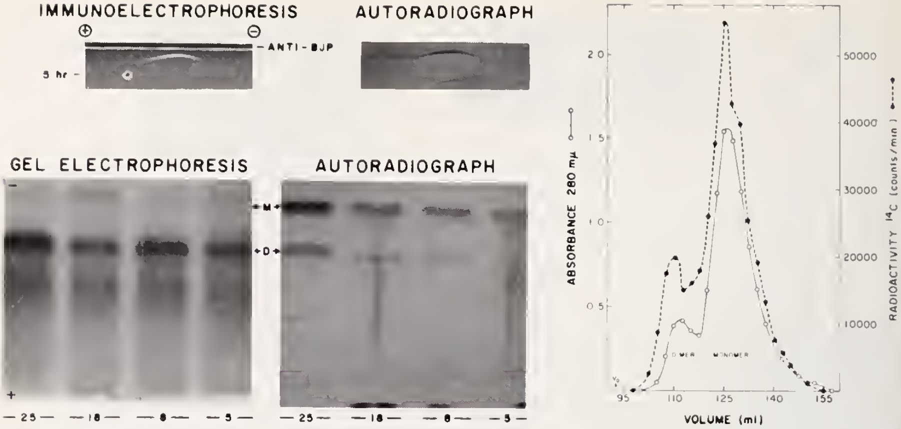

FIG. 2. Biosynthesis of Bence Jones proteins. Bone marrow cells, obtained from a patient with multiple myeloma and Bence Jones proteinuria, were incubated in a growth medium containing ^{14}C-lysine and ^{14}C-isoleucine. The extracellular fluid was harvested after 5, 8, 18, and 25 hours incubation at 37°C. UPPER LEFT, *Immunoelectrophoresis:* Newly synthesized Bence Jones protein was identified in all four cultures by radioimmunoelectrophoresis. The immunochemical and autoradiographic analyses of the 5 hour culture fluids are shown. The patient's urine was used as carrier protein; the antiserum trough contained an antiserum prepared against the homologous κ Bence Jones protein. The autoradiograph was developed after 15 days' exposure, and the pattern of the autoradiogram gave exact correspondence to the precipitin arc. LOWER LEFT, *Starch Gel Electrophoresis:* The 5, 8, 18, and 25 hour culture fluids were electrophoresed for 22 hours at 4°C in an 8 M urea-formate (pH 3.0) starch gel. The autoradiograph was developed after 3 days' exposure to the gel. A greater intensity in the labeled Bence Jones protein monomer, as compared to the labeled dimer, is evident. The progressive increase in intensity of both molecular forms in the four cultures is also apparent. The monomer (M) and dimer (D) are indicated. RIGHT, *Gel Filtration:* The molecular distribution of the labeled Bence Jones proteins was determined. One-tenth ml of the 18 hour culture fluid and 100 mg lyophilized urine dissolved in 3 ml of 0.15 M NaCl-0.1 M Tris-HCl buffer, pH 7.6, were applied to a 2.5 × 100 cm polyacrylamide (P-100) column. Blue dextran was added to serve as reference marker for the void volume (V_0) of the column. Each fraction of column effluent was analyzed for protein content (absorbance 280 mμ) and radioactivity. The similarity between the profile of protein distribution of the monomer and dimer urinary Bence Jones proteins and the profile of radioactivity of the newly synthesized monomer and dimer Bence Jones proteins in the culture is evident.

the reduced catabolism of Bence Jones proteins in ureteral-ligated or nephrectomized mice (16) as well as from the reduction in concentration of serum light chains after renal homotransplantation (17).

Bence Jones proteins are synthetic products of plasma cells (18, 19) and are *not* derived by breakdown or catabolism of myeloma proteins (20, 21). The synthetic origin of Bence Jones proteins has also been substantiated by our biosynthetic studies in which bone marrow cells of patients with Bence Jones proteinuria were used (22). Autoradiographic techniques were employed to identify newly synthesized Bence Jones proteins, i.e., labeled proteins in culture fluids (Fig. 2). The molecular forms of the newly synthesized Bence Jones proteins and of the urinary Bence Jones proteins were compared by gel filtration and starch gel electrophoretic techniques and found to be identical.

Immunohistologic methods have made possible the localization of the cellular site of Bence Jones protein synthesis (23). Cells obtained by aspiration

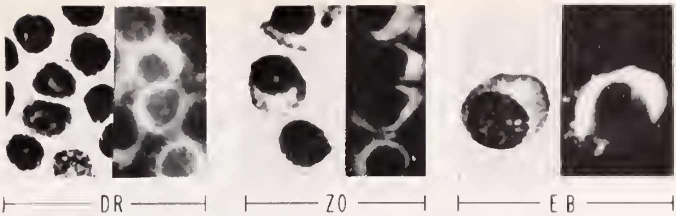

FIG. 3. Immunohistologic localization of Bence Jones protein. Giemsa-stained cells (left) and fluorescent cells (right) from three patients with Bence Jones proteinuria are shown. Positive immunofluorescent reactions were achieved only with anti-κ or -λ light chain antisera.

from the bone marrow of patients with multiple myeloma and Bence Jones proteinuria were examined by immunofluorescent techniques. The majority of tumor cells exhibited uniform and intense cytoplasmic fluorescence with type-specific anti-light chain antisera. Absorption of an antiserum with the patient's Bence Jones protein completely abolished the reaction; no positive fluorescence was obtained with anti-heavy chain (γ, α, μ) antisera. Bence Jones proteins were identified in cells which had the appearance of typical plasma cells, but in some cases the cells resembled lymphocytes (Fig. 3). This morphologic diversity among tumor cells was evident in Giemsa-stained preparations. Cells containing κ Bence Jones proteins could be distinguished immunohistochemically from cells containing λ Bence Jones proteins; however, no characteristic difference was found in the appearance of either the fluorescent or Giemsa-stained cells containing κ or λ proteins. Studies of cells, obtained from patients with Bence Jones proteinuria and whose sera contained G or A myeloma proteins or M-macroglobulins, revealed that the complete immunoglobulin molecule and the Bence Jones protein were present within the same cell. No consistent morphologic difference was apparent between cells containing both myeloma proteins and Bence Jones proteins and those cells containing only Bence Jones proteins.

The biosynthetic and immunohistochemical data have confirmed the *de novo* synthesis of Bence Jones proteins by plasma cells. Although it has been shown that heavy chains and light chains are synthesized on separate ribosomes (24, 25) and that in the myelomatosis state light chains may be synthesized in excess of heavy chains, it has not been established whether Bence Jones proteins are precursors or abortive products in myeloma protein synthesis.

Considerable information concerning the structural basis of antibody specificity has come from analyses of Bence Jones proteins. Putnam and coworkers (26) predicted on the basis of comparative tryptic peptide maps that the primary structure of a Bence Jones protein consists of a distinctive amino acid sequence, unique to each protein, and a shared amino acid sequence, common to all Bence Jones proteins of the same type (κ or λ). The results of sequence analyses (11, 27, 28) have confirmed this prediction: The first 107 amino acids of the Bence Jones protein polypeptide chain, the amino (N)-terminal half, are characterized by variance; the next 107 amino acids,

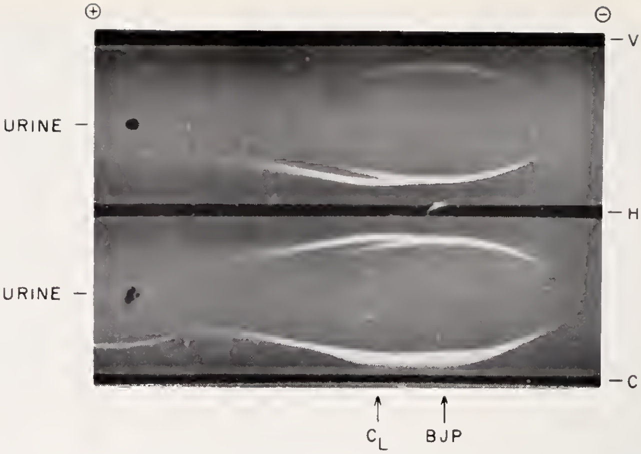

FIG. 4. Immunochemical detection of the constant half of a Bence Jones protein in urine. Urine was placed in both antigen wells and electrophoresed. The antiserum (V) in the upper trough was the anti-homologous Bence Jones protein antiserum rendered specific for V_L antigenic determinants by absorption with a heterologous Bence Jones protein. This antiserum formed a precipitin reaction only with the variant half antigenic determinants of the intact Bence Jones protein. The antiserum (H) in the middle trough was the homologous antiserum which reacts with both the constant half and variant half antigenic determinants. The C_L (indicated by the arrow) gave a reaction of partial identity with the intact Bence Jones protein (BJP) due to the reaction of antiserum H with both the V_L and C_L antigenic determinants of the intact Bence Jones protein. The antiserum (C) in the lower trough was an anti-heterologous Bence Jones protein antiserum which reacted only with the C_L antigenic determinants of the homologous protein, and thus the free C_L gave a reaction of identity with the intact Bence Jones protein.

the carboxyl (C)-terminal half, are characterized by constancy. The N-terminal and C-terminal halves are referred to as the variant half (V_L), and constant half (C_L), respectively (22).

The finding of urinary components of lower molecular weight than Bence Jones proteins, but antigenically related to Bence Jones proteins, has been of special significance (29–34). The components have been recognized immunochemically and physicochemically in approximately one-third the number of patients with both multiple myeloma and Bence Jones proteinuria (30) and have represented as much as 40 percent of the amount of urinary protein (35). Immunochemical (30) and structural comparisons (36, 37) of the smaller urinary components with the homologous Bence Jones protein have revealed a more frequent correspondence to the V_L than to the C_L of the Bence Jones protein[2]. Correspondence to the C_L of the Bence Jones protein (Fig. 4) has been demonstrated in some cases (22, 30, 31). The infrequency of the detection of C_L-related components has been attributed to the greater susceptibility of C_L to proteolysis (22, 35). The occurrence of V_L or C_L in urine has no dependence on the Bence Jones protein antigenic type, degree of proteinuria, or the status of renal function. In an individual patient the ratio of V_L or

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[2] The presence of *both* the V_L and the C_L of a Bence Jones protein has been detected in the urine of one patient, and in another patient a Bence Jones protein and C_L have been detected in the serum (22).

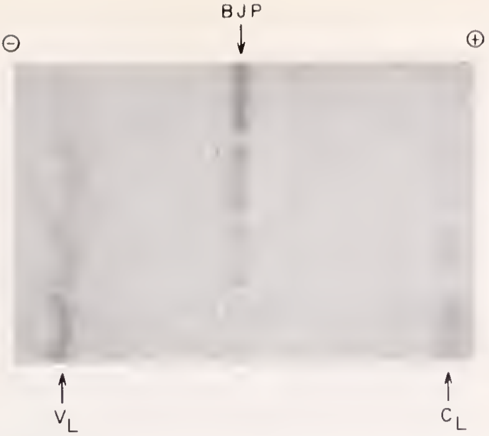

FIG. 5. Enzymatic cleavage of a κ Bence Jones protein into variant half and constant half. A Bence Jones protein was treated with pepsin (enzyme: protein ratio, 1:200) in 0.05 M glycine-HCl, pH 3.4, at 37°C. The enzymatic action was stopped after 4 minutes by bringing the sample to neutrality by addition of 1 M Tris base (Tris[hydroxylmethyl]-aminomethane). The treated sample was compared with the untreated Bence Jones protein by alkaline starch gel electrophoresis (0.05 M glycine, 0.005 M NaOH, pH 8.8). The intact Bence Jones protein (BJP), the variant half (V_L), and constant half (C_L) are indicated.

C_L to the intact Bence Jones protein remains constant, but may decrease after initiation of therapy (30, 31, 35, 37). The urinary components corresponding to the V_L or the C_L of a Bence Jones protein may result from catabolic (22, 35) or synthetic (22, 38) processes[3].

The occurrence of V_L or C_L in urine suggested to us the possibility that the peptide bond in the "switch" region linking the two halves of the Bence Jones protein polypeptide chain is particularly susceptible to proteolysis. Indeed, we (22) have shown that Bence Jones proteins can be specifically cleaved into V_L and C_L by a proteolytic factor present in urine and in a kidney extract. Cleavage of Bence Jones proteins as well as light chains of immunoglobulins into constituent halves was achieved by several types of endopeptidases (Fig. 5). The ability to cleave specifically Bence Jones proteins permitted the isolation of V_L and C_L and made available material for studies on each half.

The Bence Jones protein was initially recognized in urine because of the unique thermal property of first precipitating upon heating and then redissolving upon boiling (1, 5)[4]. Our (22) studies utilizing isolated V_L, C_L, and intact Bence Jones protein indicated that the molecular basis for the thermal behavior of Bence Jones proteins is localized to the V_L. A comparison of the properties of the V_L and the C_L with those of the intact protein should provide additional information concerning the contribution of each half to the properties expressed by the whole molecule. Of particular importance will be ascertaining whether properties characteristic of the whole molecule are a function of the V_L or the C_L *singly* or require *both* for expression.

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[3] The urine of normal individuals contains free light chains with physicochemical and immunochemical properties identical to Bence Jones proteins (39), and smaller components antigenically related to the light chain have been detected in the urine of normal individuals (40).

[4] The physicochemical basis of this phenomenon, extensively studied by Putnam and coworkers (41, 42) has been attributed to changes in non-covalent bonding and alterations of the tertiary structure of the Bence Jones protein.

The unique primary structure of Bence Jones proteins has been a subject of continued investigation. Genetic (Inv) factors have been demonstrated only for κ Bence Jones proteins and type K light chains (43). These factors are localized to a single amino acid substitution: Inv *(1)* proteins and Inv *(2)* proteins contain a leucyl residue, and Inv *(3)* proteins contain a valyl residue, at position 191 in the C_L (44). No genetic factors have been detected on the V_L. Multiple genetic theories have been proposed to account for both the constancy and variability in the primary structure of Bence Jones proteins (45). In addition to the allotypic site, the C-terminal peptide of the C_L contains the site responsible for covalent linkage between the light and heavy chains (46). Maximum expression of antibody specificity requires the combined heavy and light chains of an antibody molecule; this specificity appears to be determined by the variant half of each polypeptide chain (47).

While the V_L of each protein is unique, certain structural homologies within the V_L of Bence Jones proteins and light chains have been apparent (48–51). The variability and constancy in the primary structure of Bence Jones proteins have been evident not only through structural studies, but also by immunochemical studies (52–56). Certain antisera developed against Bence Jones proteins have been able to detect antigenic differences among Bence Jones proteins and isolated light chains of the same type (κ or λ). Comparisons of Bence Jones proteins by immunodiffusion, inhibition of immune precipitation, and hemagglutination techniques have provided evidence for at least three classes of κ proteins and two classes of λ proteins (55). In the case of κ Bence Jones proteins, these differences were not related to the genetic (Inv) type.

Recently, we obtained an antiserum which was unique in the fact that it had strong specificity for κ antigenic determinants not recognized by other anti-κ Bence Jones protein antisera (57). This antiserum, prepared against a κ Bence Jones protein possessing an N-terminal glutamyl residue, had specificity for κ Bence Jones proteins with glutamyl as N-terminal, and further distinguished into two classes κ Bence Jones proteins with aspartyl N-terminal residues. The three classes were designated as κ_{glu}, $\kappa_{\text{asp II}}$, and $\kappa_{\text{asp I}}$ (58) (Fig. 6). These three classes could be detected directly on intact type K immunoglobulin molecules. The classification of a large group of κ Bence Jones proteins, G, A, and D myeloma proteins, and M-macroglobulins (Waldenström) is shown in Table I.

Milstein (49) has made extensive comparisons of sequence data on the V_L of κ Bence Jones proteins and has defined three basic sequences. Proteins in his sequence III have glutamyl as N-terminal, while proteins in both sequences I and II are characterized by aspartyl N-terminal residues. Structural analyses by Niall and Edman (50) defined two classes of κ-chains, κ_{Smi} (N-terminal glutamyl), and κ_{Tra} (N-terminal aspartyl). Our antiserum readily dis-

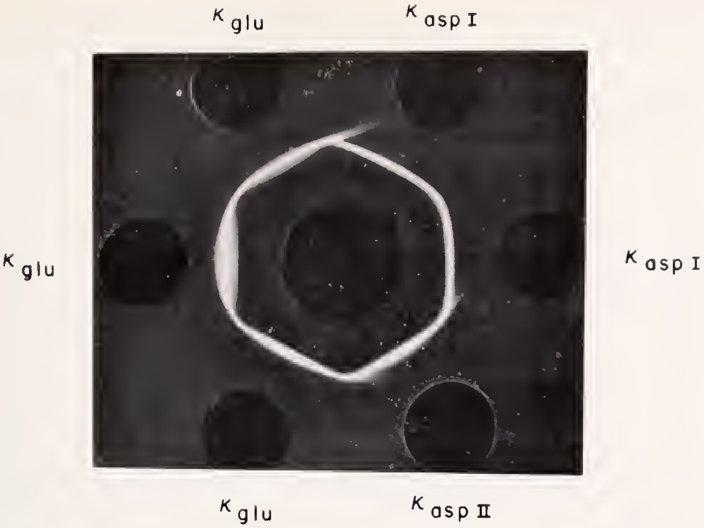

FIG. 6. Immunochemical detection of three classes of κ Bence Jones proteins. The middle antiserum trough contained an antiserum prepared against a κ Bence Jones protein possessing an N-terminal glutamyl residue. The antiserum was made specific for type K light chain determinants by absorption with urine containing λ Bence Jones protein. The specificity of the antiserum is evident from the precipitin reactions. The three κ_{glu} proteins form a precipitin line of identity and spur over the $\kappa_{asp\ I}$ and $\kappa_{asp\ II}$ proteins; the differentiation of the κ_{asp} proteins is evident by the spur of the precipitin band of the $\kappa_{asp\ II}$ protein over the precipitin band of the $\kappa_{asp\ I}$ protein.

TABLE I

Classification of κ Bence Jones Proteins and Type K Immunoglobulins

	BJP*	IgG†	IgA‡	IgD**	IgM††
$\kappa_{asp\ I}$	25	31	9	1	1
$\kappa_{asp\ II}$	8	14	3	0	1
κ_{glu}	13	15	4	0	9

* BJP = Bence Jones protein.
† IgG = G myeloma protein.
‡ IgA = A myeloma protein.
** IgD = D myeloma protein
†† IgM = M-macroglobulin.

tinguished between samples from each class and further differentiated the κ_{Tra} class proteins into two classes[5]. The concordance between immunochemical differentiation of proteins and differentiation by structural analyses suggests the importance of immunochemical methods as a means for the rapid differentiation of proteins as well as for the localization of these differences[6].

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[5] Representative samples of each κ class kindly supplied by Dr. P. Edman.

[6] Bence Jones proteins representative of basic kappa-chain sequences I, II, and III were made available, by Doctors C. Milstein, F. W. Putnam, and H. G. Kunkel. Immunochemical comparisons of these Bence Jones proteins with proteins of our three κ-chain classes revealed that proteins of basic sequences I, II, and III would be classified as $\kappa_{asp\ I}$, $\kappa_{asp\ II}$, and κ_{glu}, respectively.

While the significance of the Bence Jones protein has become apparent only in the last decade, the full significance of information which can be obtained from studies on this molecular entity remains to be realized. Thus, the Bence Jones protein continues to be an invaluable source of protein for studies designed to elucidate the structural and genetic control of antibody specificity.

Acknowledgments

I wish to express my appreciation to my former "Chief," Dr. Alexander B. Gutman, for his training and guidance during my internship and residency at The Mount Sinai Hospital, and to Doctors John L. Fahey, Henry G. Kunkel, and Miss Carla L. McLaughlin, with whom these studies represent a collaborate effort.

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Metabolic Pathways of Bile Acid Formation in the Rat

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Although it has been recognized that the bile acids are derived from cholesterol (systematic names for the compounds described are: cholesterol, cholest-5en-3β-ol; 26-hydroxycholesterol, cholest-5ene-3β, 26-diol; cholic acid, 3α, 7α, 12α-trihydroxy-5β cholanoic acid; chenodeoxycholic acid, 3α, 7α-dihydroxy-5β cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid; muricholic acids, 3α, 6β, 7α-trihydroxy-5β cholanoic acid and 3α, 6β, 7β-trihydroxy-5β cholanoic acid), the metabolic pathways for their synthesis have not been defined. One pathway, beginning with 7α hydroxylation of cholesterol, has been studied intensively (1) in the rat. Another pathway, beginning with C-26 hydroxylation, has also been reported to occur (2). Although cholic acid, a bile acid formed in rat liver, was not found to be a metabolite of 26-hydroxycholesterol in early studies (2), small amounts have been identified recently (3). The major difference between these two possible pathways appears to be the proportion metabolized to cholic acid.

Since the rat synthesizes a complex mixture of dihydroxy and trihydroxy bile acids from cholesterol, considerable variation in the proportion excreted as cholic acid could occur. In order to quantitatively evaluate the contribution of 26-hydroxycholesterol to cholic acid synthesis, the proportion of each bile acid derived from cholesterol-^{14}C and 26-hydroxycholesterol-^{3}H were determined in a rat with a bile fistula.

Materials and Methods

Cholesterol-4-^{14}C (55.8 mCi/mmole) was purchased from Nuclear-Chicago. Thin layer chromatographic analysis indicated that 96 percent of the radioactivity was in the cholesterol zone. The eluate obtained from the cholesterol zone was dissolved in 0.2 ml of Tween 20 and then added to 1.0 ml of 0.9% NaCl containing 3% human serum albumin. Radiochemical purity of the eluate was established by addition of an aliquot to recrystallized cholesterol. Synthesis and recrystallization as the dibromide caused no fall in the calculated specific activity.

26-hydroxycholesterol-16-22-^{3}H was prepared by modification of the method of Scheer, Thompson, and Mosettig (4), as described previously (3). For infusion, the compound was solubilized as described above.

Bile salts were estimated quantitatively using the enzymatic method (5). Identification of the metabolites in bile was done by thin layer chromatography on silica gel, and by column chromatography on neutral alumina, activity Grade III. For reverse isotope dilution recrystallized lithocholic, chenodeoxycholic, cholic, and 3β-hydroxy-5-cholenoic acids were used. For each com-

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pound at least two derivatives and two different solvent systems were used to obtain constant specific activity. These techniques and methods of quantitation of radioactivity by liquid scintillation spectrometry have been described in detail previously (3, 5, 6).

Following surgery during pentobarbital anesthesia, as described previously (5), the bile salt pool was depleted by overnight drainage. Cholesterol (187 μg, 27.3 μCi) was given by infusion at 0.1 ml per minute for 10 minutes. Bile was collected into graduates kept in ice water and changed at intervals of 6 to 8 hours for 24 hours. After collection of a bile specimen the following morning, an infusion of 26-hydroxycholesterol at 0.003 μmole/minute was given over a 6 hour period, and three bile specimens collected, each of 2 hours' duration. The study was terminated at the end of the infusion.

Results

The changes in bile flow and bile salt excretion rates following bile duct cannulation are shown in Table I. There was little fluctuation during the course of the study and the infusion of cholesterol and 26-hydroxycholesterol did not significantly alter bile flow or bile acid excretion.

The excretion rate of radioactivity was prompt and sustained. The fall in ^{14}C excretion rate is consistent with continual dilution of the cholesterol pool by newly synthesized cholesterol. Less than 10 percent of the ^{14}C and ^{3}H in bile was extractable from alkaline solution into ethyl acetate, indicating that the bulk of the radioactivity is in the form of acids.

The results of the analysis of bile specimen #6, obtained just prior to the infusion of 26-hydroxycholesterol, and of combined bile specimens #7 and #9 are shown in Table II.

During the infusion of tracer amounts of 26-hydroxycholesterol there was

TABLE I

Bile Flow and Bile Salt Excretion Rate in a 369 gm Male Rat before and during the Infusion of Cholesterol-^{14}C and 26-Hydroxycholesterol-^{3}H

Bile #	Time (minutes)	Flow Rate (mg/minute)	Excretion Rate		
			Bile Salt (μmole/minute)	^{14}C (cpm/minute)	^{3}H (cpm/minute)
1	1,440	16.5	0.13	—	—
2*	360	17.5	0.09	—	—
3	360	21.7	0.11	6,750	—
4	480	20.9	0.12	4,525	—
5	60	21.4	0.11	3,670	—
6	60	20.7	0.11	3,440	—
7†	120	21.7	0.10	3,050	12,500
8†	120	19.5	0.09	2,930	13,950
9†	120	21.9	0.10	3,050	12,500

* 10 minute infusion of cholesterol during this period.

† 26-Hydroxycholesterol infusion.

TABLE II

Identification of Radioactivity in Bile by Column Chromatography and Reverse Isotope Dilution

Reference	Percent of Radioactivity from Column in Reference Zone			Percent of Zone Identified as Bile Acid by Reverse Isotope Dilution		
	^{14}C Bile #6	^{14}C Bile #7 and #9	^{3}H Bile #7 and #9	^{14}C Bile #6	^{14}C Bile #7 and #9	^{3}H Bile #7 and #9
3β-hydroxy-5-cholenoate	1.3	0.9	8.1	0.02	0.07	3.4
Lithocholate	0.4	0.3	22.9	0.08	0.05	1.3
Chenodeoxycholate	19.7	26.2	36.9	18.6	24.0	29.8
Cholate	66.5	67.0	19.7	58.2	54.3	2.2
Unidentified	12.3	5.6	12.4	22.8	21.6	63.3

little change in the pattern of excretion of ^{14}C. A little more than half of the radioactivity was found in cholic acid. Most of the unidentified radioactivity was found in the cholic acid zone, and this presumably represents the muricholic acids. Thin layer chromatography in several systems of the radioactivity in this zone was also consistent with the presence of muricholic acids.

Chenodeoxycholate represented 18 to 24 percent of the ^{14}C radioactivity in the bile and 30 percent of the ^{3}H in bile. Since most of the radioactivity attributable to 26-hydroxycholesterol could not be identified by reverse isotope dilution, chenodeoxycholate represents the preponderant bile acid identified by this technique. However, it is apparent that much of the unidentified radioactivity is present in the cholic acid zone. By reverse isotope dilution only 2 percent is accounted for by cholic acid. Presumably the remainder represents muricholic acids.

Radioactivity is also present in the monohydroxy bile acid zones (lithocholate and 3β-hydroxy-5-cholenoate). Proportionally more is derived from 26-hydroxycholesterol than from cholesterol. The marked fall in the radioactivity that occurs when reverse isotope dilution is done with the reference bile acids indicates that the bulk of the radioactivity in the monohydroxy zones has not been identified.

Discussion

The purpose of this study was to evaluate the metabolic pathways a molecule resembling cholesterol, except for a partially oxidized side chain, would follow in rat liver. To derive information from this study, it must be assumed that both the administered cholesterol and 26-hydroxycholesterol mix completely with their respective pools and are metabolized in a manner identical to the endogenously synthesized compounds. This assumption is implicit in all studies of cholesterol turnover and metabolism. The occurrence of 26-hydroxycholesterol in atheroma (7) and its synthesis from cholesterol *in vitro* (2) suggests an endogenous pool with which mixing can occur.

Since the proportion of cholate that is synthesized by the rat can vary, it is important to know the synthesis rate of the different bile acids at the time a particular intermediate is evaluated. It is also important not to alter significantly the size of the metabolic pool of intermediates.

To achieve the above conditions, the animal was given ^{14}C-cholesterol 24 hours prior to the study to allow for equilibration with the endogenous pool. The total bile salt excretion rate was determined just prior to the infusion of 26-hydroxycholesterol, and can be assumed to be the rate of synthesis of new bile salt since the pool had been depleted by biliary drainage for 44 hours. The infusion rate of 26-hydroxycholesterol was chosen to be 3 percent of the bile salt excretion rate. This could not represent more than a 3 percent change in the pool size of intermediates in the liver. No marked change in the pattern of ^{14}C excretion as bile salt occurred during the infusion, indicating that the method of solubilization and administration did not alter bile salt synthesis. It seems reasonable therefore to compare the pattern of 26-hydroxycholesterol metabolism with that of cholesterol metabolism.

The proportions of chenodeoxycholate derived from cholesterol and 26-hydroxycholesterol are quite similar. Since deoxycholate (3α, 12α-dihydroxy-5β cholanoic acid) is not considered to be derived from cholesterol, it is reasonable to assume that 7α hydroxylation precedes and does not follow 12α hydroxylation. If 12α hydroxylation of cholesterol and 26-hydroxycholesterol had occurred to a similar extent, then the proportions of cholate formed from each precursor would also have been the same. However, it is apparent that there is much less synthesis of cholate from 26-hydroxycholesterol. In lieu of 12α hydroxylation to form cholate, it appears that there has been considerably more 6β hydroxylation to form muricholate. Since trihydroxy bile acids consisting of hydroxylation at the 3α, 6β, and 12α position have not been found as significant metabolites of cholesterol in the rat, it appears that 6β hydroxylation inhibits 12α hydroxylation. The most reasonable conclusion from this simultaneous comparison of cholesterol and 26-hydroxycholesterol metabolism in the rat is that the compound with the partially oxidized side chain undergoes more extensive 6β hydroxylation, leading to a decrease in cholic acid formation.

Studies concerning bile acid synthesis in the rat began (8) before it was recognized that this species could synthesize bile acids other than cholate and chenodeoxycholate (9). The bile acids occurring as a result of 6β hydroxylation of the steroid ring were termed muricholic acids when they were also found to occur in the mouse. Because infusion of chenodeoxycholate into the rat led to excretion of muricholates, Bergstrom (8) preferred to consider them as derivatives of chenodeoxycholate rather than having their origin in cholesterol. Although chenodeoxycholate may be an intermediate in their formation, the presence of 3α, 6β-dihydroxy-5β cholanoic acid in rat bile (6) indicates that hydroxylation at the 7α position may not be mandatory and may follow 6β hydroxylation. Their origin from cholesterol in the bile fistula animal is also consistent with considering the muricholic acids as primary bile acids in the rat.

The rat has been the principal animal for the study of bile acid metabolism.

Their capacity to survive for prolonged periods of time following bile duct cannulation and the ease of the surgical procedure in the absence of the gall bladder has probably encouraged their use. However, the existence of pathways of bile salt metabolism in the rat that do not occur in man or in other species, such as the hamster (6), may limit their usefulness in evaluating the metabolic pathways for cholesterol metabolism that are relevant to man.

Summary

A dual isotope study using cholesterol-^{14}C and tracer amounts of 26-hydroxycholesterol-^{3}H has been done in a rat with a bile fistula. Both compounds have been found to be metabolized to chenodeoxycholic, cholic, and muricholic acids. Although the proportion of each precursor metabolized to chenodeoxycholate is approximately the same, considerably less cholate and more muricholate is synthesized from 26-hydroxycholesterol. The difference is attributable at least in part to the alternate pathway of 6β hydroxylation that occurs in the rat.

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The Gastric Mucosal Barrier: Physiological and Clinical Considerations

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The inherent resistance of the gastric mucosa to autodigestion has occupied the attention of physiologists for more than two centuries. John Hunter (1), on observing postmortem gastric autolysis, suggested the presence of a "living-principle" which protects the stomach during life; this, he proposed, derives from the blood circulating through the tissue. Virchow's (2) addition to this hypothesis proposed the back-diffusion of gastric acid into the mucosa and its neutralization there by alkaline blood circulating in the area. In recent years the function of the gastric surface epithelium has been the subject of a considerable amount of study and speculation. Physiological investigations have established that gastric surface mucosa presents a "barrier" to the back-diffusion of H+ from the gastric lumen, after HCl has been secreted by the gastric glands or instilled from exogenous sources; the restriction of concomitant migrations of Na+ and K+ across this membrane is also well documented. These observations form the basis of an hypothesis which holds the function of the gastric surface epithelium to be the preservation of the intralumenal acidity, by means of the unusual ability of the mucosa to resist back-diffusion of acid secreted by the gastric glands, thereby also resisting acid-peptic ulceration. An opposing school of thought (3) sees the function of this membrane to be the reduction of intralumenal acidity by affording a site and mechanisms for the rapid and safe passage out of the lumen of H+ of strong acids, such as secreted HCl.

In his formulation of an hypothesis regarding the intragastric mechanisms responsible for the modification of the primary gastric acid secretion, Hollander presumed the gastric juice to be composed of: (1) parietal cell secretion containing HCl at isosmotic concentration, and no other ions; and (2) an alkaline component comprised of mucus, enzymes, and a transudate of interstitial fluid. These components were conceived of as relatively invariant physiological entities and all variations in intragastric acidity and electrolyte composition were presumed to result from neutralization and dilution of the parietal component by the non-parietal component. No allowance was made for the possibility that any major intragastric alteration in the composition of the primary acid secretion resulted from diffusion or ion-exchange across the surface epithelium. When Hollander (4) formally stated his concept of the gastric lining as a two-component barrier, he drew attention to the adherent layer of mucus and to the surface epithelial cell layer, indicating their combined function as primarily protection. Many premises of the Hollander multi-

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component secretion hypothesis and the mucus-mucosal barrier hypothesis receive substantial support from a large body of experimental evidence. One major premise of the former—the absence of Na+ and K+ from parietal cell secretion—is, probably, only partly true. Most evidence indicates that at least a little K+ is part of the primary gastric acid secretion. This question has been the subject of considerable investigation in this laboratory (5-7); it remains controversial. Our (8) recent observation of relatively high rates of K+ output by pyloric gland area (PGA) mucosa (non-acid secretory) in response to contact with HCl on its lumenal surface clarifies some, but not all, of the anomalies noted in regard to gastric K+ migrations during acid secretion. Other incompatibilities in a variety of experimental observations also may indicate that the barrier concept is not entirely correct, and that ionic movements across the surface epithelium account for some proportion of the variations of composition noted in gastric juice as it is collected from the lumen. Because of the free permeability of the adherent mucus layer to secreted acid passing from gastric pits and foveolae into the lumen, and in view of the free diffusion of HCl through concentrated films of gastric mucus *in vitro* (9), attention has largely turned to the function of the surface epithelial cell layer as a barrier to H+ and other ionic diffusions.

The early observations of Teorell (3) on the rapid disappearance of H+ (without an increase in volume) from HCl instilled into ligated cat stomachs, led him to conclude that this loss occurred via exchange diffusion for Na+ across the surface mucosa. He argued that the total volume would have increased had a significant reduction in acidity resulted from neutralization. The small volumes of HCl used in these comparatively large stomachs without an indicator to measure residual volume may have influenced the data. It is also noteworthy that these observations were made in whole stomachs which included PGA mucosa, whereas the majority of evidence in support of the barrier concept was obtained in isolated systems employing oxyntic gland area (OGA) exclusively. Subsequently, using larger volumes of instillate, Teorell (10) again concluded that H+ of strong acids diffuse out of the stomach quite easily; however, the results were somewhat different from the initial report. Although data were not detailed, "larger volumes showed very little decrease in acidity during the time of the experiment." This may, indeed, have been an early demonstration of the barrier function of the surface mucosa. An interesting aspect of this latter report was the observation that weak acids disappeared much more rapidly than strong acids, a phenomenon ascribed to the entry of un-ionized acid molecules via paths (solution in the cell membrane) other than those open to ions. The inflammatory effects upon the mucosa of the entry of these weak acids were noted, but better appreciation of their significance awaited the work of later investigators (11). The experiments of Elliot, Rosholm, and Obrink (12) on intact man also show H+ disappearance without volume change, and are much quoted in support of Teorell's diffusion hypothesis; however, the study fails to account for pyloric losses of instilled acid, residual volumes, or acid secreted, and so is difficult to interpret. More recently, similar acid instillation experiments have been repeated in intact man (13), refined by the addition of a dilution indicator to estimate pyloric losses. In contrast to the foregoing, the authors found a significant increase in volume after instillation of isotonic HCl. Nevertheless, they agreed that intragastric acidity was, in large part, reduced by exchange-diffusion across the mucosa. Their argument is weakened, however, by failure to account for residual volumes, and the resultant inability to calculate net fluxes.

Documentation of the presence of a barrier of H+ and other ionic diffusions at the level of the surface epithelium has been extensive. Na+ insorption from the gastric lumen from neutral solutions is quite slow for the entire stomach (PGA as well as OGA) (14–16). In the presence of acid in the lumen (either secreted or instilled), this restriction becomes virtually complete in the OGA, but is unaffected in the PGA. The restriction of Na+ insorption is dependent upon its ratio to H+ present, the latter being greatly favored, so that Na+ must outnumber H+ by a ratio of 10:1 before Na+ gains access to what may be a common mechanism of transport. K+ insorption suffers similar restriction from neutral solutions, but is unaffected by lumenal H+, and so may have a mechanism or site of transport separate from Na+ and H+. Migration of Na+ from blood to lumen is also greatly restricted, and is considered to result from migration down its electrochemical gradient. The rate of exchange-diffusion of H+ for Na+ across the surface mucosa must be very slow, indeed, especially for OGA mucosa. Entry of Na+ into isotonic HCl placed in the gastric lumen was only 0.23 mEq/hour in *vivo-vitro* (intact blood-supply) chamber preparations of canine OGA (17) and 0.36 mEq/hour in vagally denervated canine OGA pouches (11). Furthermore, if exchange-diffusion normally plays an important role, Na+ output should be greater into acid solutions than into neutral ones; however, this is not the case (8, 11).

H+ is lost from the gastric lumen at very slow rates in isolated OGA preparations. Vagally denervated *vivo-vitro* chamber preparations of canine OGA indicated H+ loss from instilled isotonic HCl solutions to be only 0.35 mEq/hour (10 percent/hour) (18), and this rate is remarkably stable over prolonged periods of observation. Similar rates have been reported in vagally denervated canine OGA pouches (11), i.e., 0.2 mEq/hour (5 percent/hour). Recent observations (19) indicate that higher rates of H+ back-diffusion may occur in intact man: 2.2 mEq/15 minutes (6.9 percent of the instillate). Na+ and K+ outputs during acid instillation in this study were also relatively high: 3.0 mEq/15 minutes and 0.5 mEq/15 minutes. Some of the differences between these studies in intact humans and those done in isolated OGA systems may be related to the larger surface area, the intact vagal innervation, or to the repeated renewal of the instillate with relatively large volumes of HCl (160 mEq/L) every 15 minutes. Furthermore, some of the PGA may have been in contact with the instilled HCl in the human experiments.

In order to evaluate further the role of the PGA we (8) have performed isotonic HCl instillations in denervated canine PGA pouches and compared them to OGA pouches of the same stomachs (each stomach has its own barrier characteristics). Back-diffusion of H+ and concomitant Na+ gain was found to be 2.3 times greater, and K+ gain 8 times greater in the PGA pouches (which were smaller in size than the OGA pouches). When related to surface area, H+ loss was 14 times greater, Na+ gain was 19 times greater, and K+ entry was 50 times greater with PGA pouches than with OGA pouches. These findings suggest that some of the differences between isolated OGA systems and intact stomachs may be related to the relatively increased permeability characteristics of the PGA surface epithelium. Furthermore, these permeability characteristics of the PGA may relate to its high vulnerability to acid-peptic digestion (20), to the frequency of diffuse inflammatory and metaplastic changes in the PGA in peptic ulcer disease (21), and to its physiological functions (i.e., acid-inhibition of gastrin release). On the other hand, one notes the relatively small size of the PGA (50 cm^2, or 10–15 percent of the gastric surface) in normal stomachs, and its high level of motor activity, which makes it unlike the reservoir of the corpus and fundus, so that gastric contents pass through the PGA (antrum) quickly. These factors raise questions as to whether the increased permeability of the PGA has physiological significance in modification of acid secreted by the proximal stomach.

One method of studying the surface epithelium has been to alter it by a variety of means and to study the functional and anatomical result. Some of these alterations result in obvious anatomic abnormalities, and so are easy to classify as being "abnormal" as well. On the other hand, important alterations in surface epithelial permeability characteristics have been observed after exposure of the mucosa to such substances as hypertonic sugar solutions similar to those in the normal diet and to normal human bile, such as might regurgitate into the stomach under normal or pathological circumstances. Disruption of the biochemical gastric mucosal barrier, and the consequences thereof, have occupied the attention of a number of investigators, and have yielded important information. The general findings have been as follows: (1) The breaking of the mucosal barrier causes no apparent changes in net water movement and electrolyte fluxes when neutral solutions are in the gastric lumen; (2) When HCl solutions contact mucosas with impaired barrier function, there is an increased rate of loss of H+ from the lumen, with concomitant increase output of Na+, K+, and water from (or through) the mucosa; (3) This increased rate of penetration of lumenal H+ into the mucosa, and not the diminished barrier per se, appears to be the crucial factor in causing tissue damage, local histamine release, bulk flow of interstitial fluid into the lumen, bleeding, and ulceration; and (4) Most of the changes noted after breaking the barrier are rapidly reversible, especially with neutral solutions in the lumen.

Experiments in our laboratory utilizing the *vivo-vitro* chamber device and canine OGA mucosa indicate that parenteral acetazolamide causes a marked decrease in the ability of the surface epithelium to maintain acid solutions within the gastric lumen (18). After acetazolamide, there is an increased rate of H+ loss from the lumen (from 3.5 mEq/hour in controls to 1.1 mEq/hour); concomitant Na+ entry into the lumen is increased threefold, and water output twofold. These changes are followed by gross mucosal damage, with edema, ulceration, and hemorrhage. Acetazolamide given to similar preparations with neutral or buffer solutions in the lumen results in no change in water or electrolyte movement, nor in any gross or microscopic damage. It was concluded that acetazolamide, possibly by inhibiting carbonic anhydrase in the surface mucosa, impairs the barrier function of this membrane, allowing a rapid rate of lumenal H+ back-diffusion, with subsequent tissue damage and leakage of Na+ and water into the lumen. It was suggested that at least part of the "inhibition" of acid secretion previously attributed to acetazolamide actually results from the loss of secreted acid by back-diffusion across a functionally altered surface epithelium before it can be collected and titrated.

Davenport and his colleagues (11, 22–25) have afforded further quantitative information on the movement of H+, Na+, K+, Cl−, and water across surface epithelium with normal or impaired barriers with various lumenal solutions. They indicate that un-ionized and fat soluble substances may rapidly diffuse through the surface epithelium, and that certain of these have the ability to alter the barrier function of the surface mucosa as they diffuse across it (and for some time thereafter). Contact of the mucosa with H+ after absorption of such compounds as acetic acid, propionic acid, acetylsalicylic acid, and ethanol results in increased transmucosal flux of H+, Na+, and K+. Similar changes were noted after contact of the OGA mucosa with dog bile, sodium taurocholate decyl sulfate, and urea (26). The (even moderately) increased movement of H+ back through the surface mucosa is associated with signs of injury and disappearance of the normal transmucosal potential difference (PD). When glycine is placed within the gastric lumen to make secreted H+ less available for back-diffusion (i.e., the "glycine-trap" experiment), it can be demonstrated that this loss of PD does not result in loss of the H+ secretory ability of the mucosa. These authors conclude from these most important observations that the Na+ barrier and transmucosal PD to a large extent result from the function of cells which are necessary for the maintenance of intralumenal acidity, but not essential to the production of the HCl in the first place.

Experiments in this laboratory (18) indicated that a large proportion of the increased Na+ output from damaged mucosa was associated with net water movement into the lumen and followed the increased penetration of lumenal H+ through an altered mucosal barrier. Davenport, on the other hand, initially suggested that the increased Na+ gain and H+ loss be looked upon as an acceleration of the normally minimal equilibration across the membrane. Probably both mechanisms are involved. Increased insorption of H+ through the surface mucosa with a decreased barrier function results in increased fluid output. This fluid has been shown (24) to contain considerable amounts of glucose (normally very little appears in the gastric juice) and increased amounts of plasma protein. Davenport estimates that 30 percent of this fluid comes from interstitial fluid and that 10 percent has leaked from the plasma. The remaining 60 percent is unaccounted for, but conceivably some of it represents water associated with HCl secretion, from which H+ has been lost by exchange-diffusion for Na+ across the altered surface epithelium. Topical histamine applied to mucosas with impaired barriers results in the output of large amounts of fluid, mainly plasma, and in the stimulation of a small amount of HCl secretion (27). Normally, the gastric mucosa is not penetrated by histamine. Furthermore, it has been shown that histamine appears in the gastric venous effluent of dogs whose stomachs have been damaged by salicylates (28).

Gastric mucosal bleeding which attends higher rates of H+ back-diffusion (18) is increased when accompanied by cholinergic stimulation (26). These effects may be accentuated by both the increased motor activity and the vasodilatation associated with cholinergic stimulation; increases in motor activity are seen to occur with increased H+ back-diffusion (29), and vasodilatation may result from local histamine release caused by the tissue-damaging effects of back-diffusing H+.

It is also of great interest that contact of the mucosa with simple sugar solutions in somewhat hyperosmotic concentrations (within the range found in common components of the diet) may cause increases in the permeability coefficients of the surface mucosa (30). Different solutes may vary in their effect on the barrier, glucose being more effective than sucrose in causing this alteration. As with other substances (e.g., aspirin, acetic acid, and bile), this increased permeability is rapidly reversible. Altamirano also has demonstrated the important fact that Na+ and HCO_3- may flow into neutral solutions in the gastric lumen without significant net movement of water.

Although the surface mucosa of the PGA is normally far more permeable than that of the OGA, it too has important intrinsic barrier activity against the free diffusion of certain ions. Studies of canine PGA pouches have been carried out in our laboratory in the past few years and demonstrate that a number of substances which impair the OGA surface mucosal barrier have similar effects in the PGA.

In view of the localization of gastric ulcers in PGA mucosa (20), as well as the regular occurrence of diffuse and severe inflammatory changes confined primarily to the PGA mucosa in gastric ulcer disease (21), and the demonstration of increased bile regurgitation into the stomach in gastric ulcer disease (31, 32), the effects of human bile on PGA mucosa are obviously of special relevance. Human common-duct bile (8.7 to 9.4 mM), consisting mainly of glycine conjugates of cholic and chendeoxycholic acid and a small amount of glycodeoxycholate, produced disruption of the surface barrier in PGA canine pouches, so that H+ losses and Na+ and K+ gains were increased (33). The degree of change in the PGA barrier is less marked than after similar instillations into OGA pouches of the same stomachs. However, the normally more permeable PGA mucosa was made much more so by contact with bile. After a brief (30 minute) exposure to bile, these changes were reversible within 1 hour, despite continued contact with HCl. Whether or not repeated or prolonged exposure of PGA mucosa to bile and HCl will lead to the chronic inflammatory and metaplastic changes seen in human gastric ulcer stomach or to chronic peptic ulcer itself has not yet been determined.

Parenteral acetazolamide has also been shown to disrupt the canine PGA mucosal barrier much the same as seen in the OGA (34). The permeability of both PGA and OGA surface mucosa is moderately increased by parenteral atropine (35). This drug appears to affect the PGA and OGA somewhat differently. Permeability to H+ is increased in both areas; however, permeability to Na+ is increased to a greater extent in the OGA, but only minimally influenced in the PGA. The findings may indicate further functional differences between the surface barriers of the OGA and the PGA. Finally, one may conclude that as with acetazolamide, some of the apparent decrease in gastric secretion after atropine or vagotomy may result from a loss of secreted H+ back through an abnormally permeable surface mucosa.

Few adequate studies of surface epithelial function in human stomachs are available. We have examined the hypothesis that the abberations of secretion (as measured by standard secretory tests) noted in some gastric ulcer patients (i.e., hyposecretion of acid and increased non-parietal component) may be related to an increased rate of back-diffusion of H+ (19). Quantitative net flux data were made possible by use of an indicator (phenol red) to measure secretory gains, transpyloric losses, and residual volumes. H+ was insorbed in control subjects at somewhat higher rates than anticipated on the basis of previous experience (2.2 mEq/15 minutes). Whereas the fall in H+ concentration and entry of Na+ was significantly increased in several gastric ulcer subjects, significant increases in H+ loss were not demonstrated for the gastric ulcer group. Under the conditions of this experiment, gastric ulcer patients insorbed 2 to 8 times as much H+ as they appeared to secrete in the basal state, and several insorbed acid at a greater rate than they secreted it during an augmented histamine test. One striking finding of this study was the high rate of water output from "basal state" mucosas in contact with isotonic HCl (averaging 153 ml/hour in controls). If one partitions this water between parietal and non-parietal components on the basis of the net Na+ gain, assuming a secreted concentration of Na+ of 150 mEq/L, then about half of this net volume gain is calculated to be parietal secretion (12.4 mEq/hour). The question arises: Does contact of the gastric mucosa with HCl under the conditions described stimulate HCl secretion?

Some question has been raised regarding the validity of phenol red as a nonabsorbable dilution indicator in this type of experiment. However, in solutions of low pH, phenol red recoveries from the stomach have consistently been excellent: 95–99 percent (8, 13, 36, 37). The confusion appears to result from the finding of poor phenol red recoveries from lumenal solutions of lesser acidity or neutrality and from poor recoveries (83 percent) noted during initial instillation periods when the stomach has not been previously washed out with HCl (37).

A recent report of glycine instillation experiments in intact man (38) showed no difference in response of normal controls, duodenal and antral ulcer patients, and gastric ulcer, gastritis, and hypochlorhydria patients. The plan of these experiments was to determine if increased back-diffusion of H+ occurs, in any of the above circumstances, by "trapping" secreted H+ in the lumen with an instilled glycine buffer. Presumably, the additional H+ recovered with glycine instillation during an otherwise standard augmented histamine test, when compared to H+ recovered by the ordinary augmented histamine test, would give a measure of the rate of back-diffusion of H+. Unfortunately, the glycine instillation itself stimulates acid-secretory rates beyond the level attained with augmented histamine, making such a comparison difficult. Also, the dilution indicator used here was Cr^{51} Cl_3 , which gives poor recoveries from gastric instillates at pH 3.1 to 7.3. This report also presents the results of acid instillation experiments in the same groups of patients. The report fails to account for H+ secreted or neutralized during the course of the experiment, thereby treating the entire gastric mucosa including parietal cells and surface mucosa as a unit. The resultant net flux data fails to reflect back-diffusion across to surface mucosa, per se. Instead, it represents the sum of the separate processes of parietal cell acid secretion, neutralization by non-parietal component, and back-diffusion of H+ across the surface mucosa.

Definition of the mechanisms, rates, and circumstances of the intragastric alteration of the primary gastric secretion is fundamental both to the understanding of gastric secretory physiology, and to the clinical assessment of gastric acid secretory function. Both the investigator and the clinician depend upon analysis of the fluid which is collected from the gastric lumen and, clearly, this differs from what has been secreted by the gastric glands. Furthermore, understanding the function of the surface epithelium as a barrier to H+ back-diffusion (or, alternatively, the mechanisms whereby significant amounts of back-diffusing gastric acid are safely handled by the normal gastric mucosa) is basic to understanding the pathogenesis of peptic ulcer, in all its forms, and for providing more rational and effective management thereof.

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Pulmonary Function and Radiographic Changes in 598 Workers with Varying Duration of Exposure to Asbestos*

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Introduction

Previous investigations of pulmonary function in asbestosis have defined a pattern characteristic of interstitial pulmonary disease (1–4). The principal findings were uniform reduction in lung volumes, well preserved ventilatory function, decrease in diffusing capacity, impaired oxygenation of the arterial blood, and reduced pulmonary compliance. Evidence of airway obstruction was rarely encountered (1–3).

Of the various indices of lung function, those most frequently abnormal, and therefore most sensitive for detection of altered pulmonary function in asbestos workers, were vital capacity (1–3), diffusing capacity, and lung compliance (3, 5). Thomson, Palzer, and Smither (3) demonstrated, furthermore, that the vital capacity alone correctly detected abnormality in all but a few individuals.

In a prospective study of lung function in asbestos workers reported at the first International Conference on the Biological Effects of Asbestos, a progressive decrease in vital capacity over a decade was found (2), and the vital capacity was shown to be a more sensitive index of dysfunction than measurements of ventilatory function or arterial oxygen saturation at rest and with exercise. In addition, vital capacity correlated well with dynamic lung compliance. Since measurement of dynamic compliance did not require patient cooperation, it confirmed the validity of the vital capacity measurement and eliminated the possibility that the vital capacity impairment was due to poor patient performance (2).

Correlation between pulmonary function and radiographic findings in asbestos workers has in general been incomplete (1, 4). Of the two, altered function seems more sensitive an index of involvement than radiographic findings. The relationship, however, of both pulmonary function and radiographic abnormalities to duration of exposure to asbestos in a large group of workers has not been clearly established.

Since 1962, all asbestos insulation workers in one union group have been examined according to a specific protocol including occupational and medical history, physical examination, chest x-ray, and lung function studies. For mass

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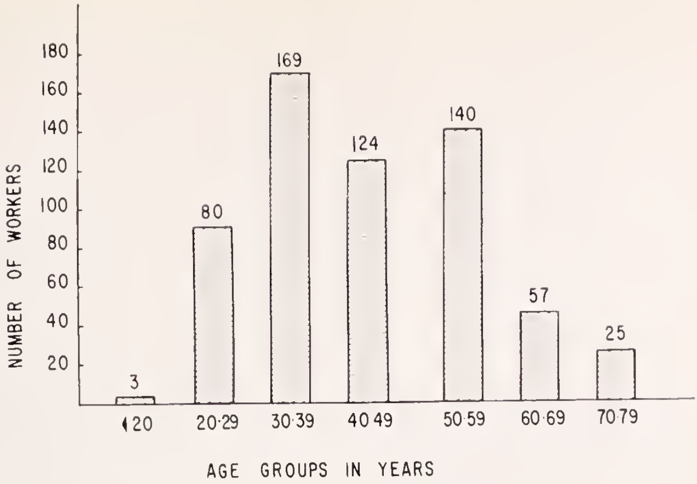

Fig. 1. Distribution of workers by age.

screening, vital capacity and 1 second forced expiratory volume (FEV_1) were employed.

Data obtained have been analyzed to evaluate the frequency and type of functional impairment. In addition, the data permitted analysis of the relative incidence of functional versus radiographic involvement, as well as the relation of both to duration of asbestos exposure.

Materials and Methods

Studies were carried out on 598 male members of an asbestos workers union. Figures 1 and 2 show their age distribution and years worked in the asbestos insulation industry. In recording the history, special emphasis was placed on duration of exposure, smoking, and symptoms, e.g., dyspnea and cough. Physical examination was done with particular reference to the presence of rales, rhonchi, clubbing, cyanosis, and signs of congestive heart failure. Chest roentgenograms were analyzed for parenchymal and pleural fibrosis and pleural calcification. Grading was categorized by a modified Saupe scale. The categories were: 0 = no abnormality; 1 = minimal fibrosis; 2 = moderate fibrosis; and 3 = extensive fibrosis. These categories were also applied to pleural fibrosis and/or calcification. Similar categorization was used for clinical indices. In an attempt to clearly define more extensive involvement (pleural or parenchymal abnormality), grades 0 and 1 were considered together, and grades 2 and 3 were considered together. Pulmonary function screening was done using a McKesson Vitalor, and the permanent record obtained was analyzed for vital capacity and FEV_1. Predicted normal values for vital capacity were derived from the tables of Bates and Christie (6), which are based not only on height and sex, but also on age. Vital capacity below 75 percent of predicted, and FEV_1 percent below 70 percent of total vital capacity were considered abnormal.

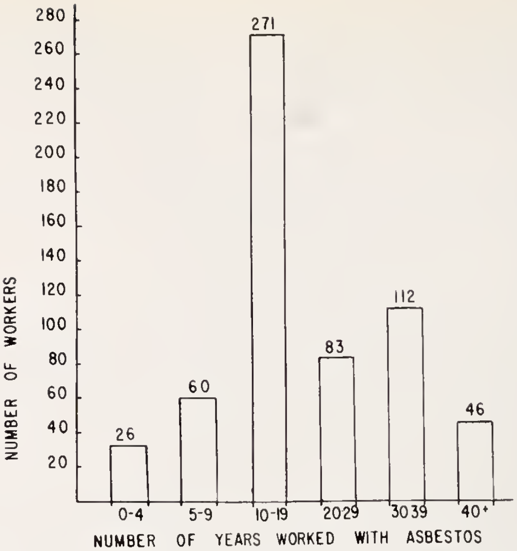

FIG. 2. Distribution of workers by number of years in asbestos insulation work.

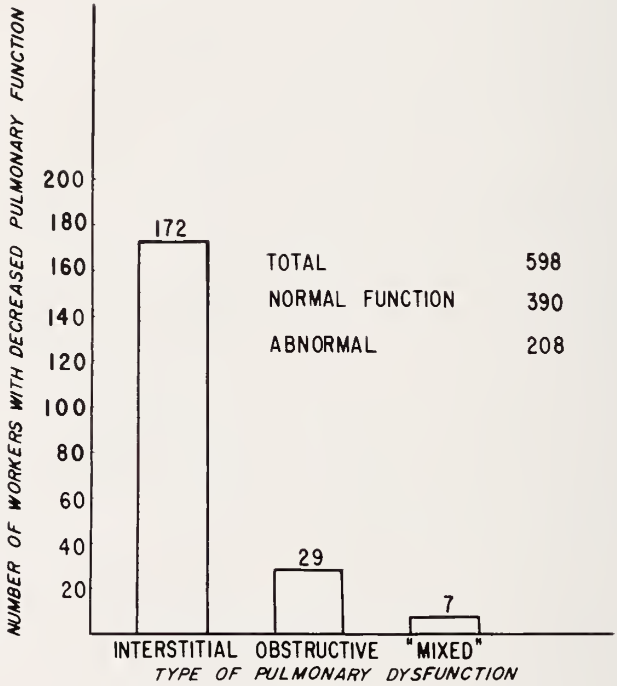

FIG. 3. Distribution of the types of functional abnormality in those 208 workers with decreased function.

Results

PULMONARY FUNCTION

Of the 598 workers studied, vital capacity was normal in 390 (65 percent) and decreased in 208 (35 percent). Of these 208, 172 had a normal FEV_1 as found in interstitial pulmonary disease. Twenty-nine had a reduction of both vital capacity and FEV_1, a finding compatible with obstructive lung disease. In seven studies, there was more marked decrease in vital capacity than in FEV_1, suggesting mixed interstitial and obstructive pulmonary disease (Fig. 3).

RADIOGRAPHIC FINDINGS

Of the 598 workers, 498 (83%) had chest x-rays in categories 0 or 1, 53 had moderate (category 2), and 10 advanced pulmonary fibrosis (category 3). Extensive pleural fibrosis was found in 30 workers (5 percent), and pleural calcification in 36 (6 percent) of these men.

In all, 100 workers (17 percent) had abnormal chest x-rays of categories 2 and 3. Of these, 45 had parenchymal fibrosis alone, 37 had only pleural lesions, and 18 had both parenchymal and pleural changes (Fig. 4).

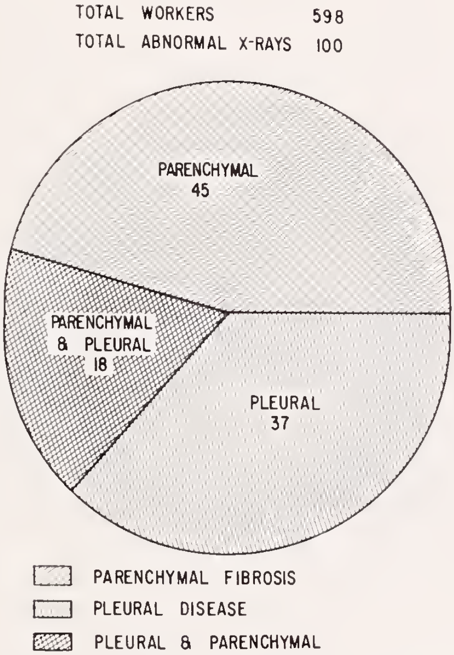

FIG. 4. Radiographic patterns in the 100 significantly abnormal x-rays. Pleural = pleural fibrosis and/or calcification only; parenchymal = parenchymal fibrosis only; and parenchymal and pleural = both parenchymal and pleural involvement.

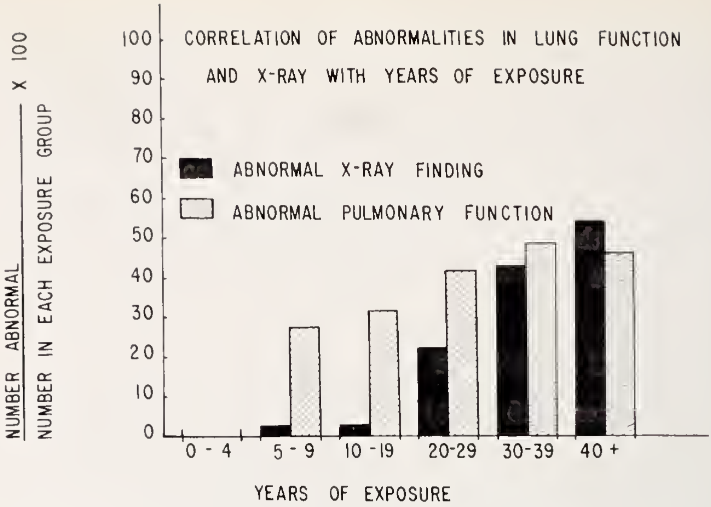

FIG. 5. Incidence of abnormal pulmonary function and significantly abnormal radiographic pattern related to years of exposure.

CLINICAL FINDINGS

In this group of ambulatory workers, symptoms and signs of pulmonary disease were not excessive. Dyspnea of grades 2 or 3 was present in only 34 workers (6 percent), and cough of these grades in 64 (11 percent). Rales in significant numbers were the most common physical abnormality encountered, occurring in 75 workers (13 percent). The remaining physical findings (in grades 2 or 3) included cyanosis in 8 workers (1 percent); clubbing in 13, (2 percent) and rhonchi in 22 (4 percent). None of the workers in this series had evidence of cor pulmonale at the time of study.

Smoking habits of the 598 workers were analyzed: 20 percent never smoked, and of the remainder all were still smoking or were ex-smokers. In these groups, regardless of the smoking history, there was no significant difference in lung function. It is relevant that if smoking produced lung function change, it would be obstructive in nature, a finding which was infrequent in this series.

CORRELATIONS

Functional and radiographic abnormality related to years of exposure: Functional abnormality related to years of exposure to asbestos is shown in Figure 5. Of the 25 workers employed in the industry for less than 5 years, none had significant pulmonary dysfunction. Abnormal pulmonary function appeared in 27 percent of the workers with 5 to 9 years of exposure, in 32 percent of those with 10 to 19 years of exposure, and in more than 40 percent of those with greater than 20 years' exposure.

No extensive (grades 2 and 3) radiographic abnormalities were detected in workers exposed less than 5 years. Indeed, such abnormalities did not appear in appreciable numbers until after 20 years of exposure (Fig. 5). It is apparent that significant functional abnormalities appear much earlier (5–9 years of exposure) than extensive radiographic abnormalities. After 30 years of exposure, the incidence of abnormal lung function and abnormal chest x-ray were approximately the same.

An overall view of the greater sensitivity of functional, as compared with radiographic, change is seen in Figure 6 and Table I. Of 250 workers with either extensive radiographic and/or pulmonary function abnormalities, 60 percent had a decreased vital capacity with normal or grade 1 abnormal x-rays, while only 16.8 percent had grade 2 or 3 x-ray abnormalities without functional impairment. Of these 16.8 percent (42 cases), only 24 cases had parenchymal involvement (with or without pleural involvement). Thus, it is evident that in 100 cases with grade 2 or 3 parenchymal fibrosis by x-ray, 24 cases (24 percent) had normal vital capacity. The overlap of 23.2 percent represents the parallel trend of grossly abnormal x-ray and pulmonary function which takes place with protracted exposure to asbestos.

Discussion

Pathologically, asbestos particles produce interstitial pulmonary fibrosis with the physiologic changes present in the "alveolar-capillary block" syndrome (1, 2, 7–11).

In a study of the discriminant value of 11 pulmonary function indices,

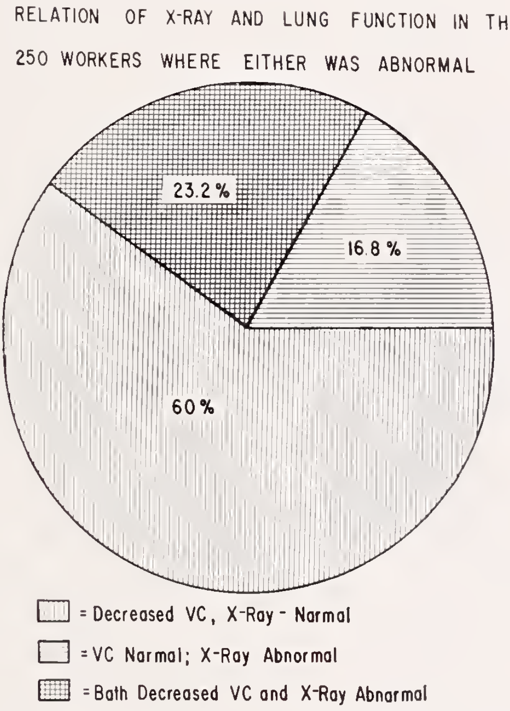

Fig. 6. Relationship of roentgenogram and pulmonary function in 250 workers in whom either was abnormal.

TABLE I

Analysis of Relationship between Grade 2 or 3 Radiological Abnormality and Decreased Vital Capacity in 250 Asbestos Workers in which Either or Both Were Present

	Number	Percent of Total of 250 Cases
Subjects with Decreased Vital Capacity	208	83.2
Radiological abnormality of grades 2 or 3		
parenchymal	29	11.6
pleural	19	7.6
parenchymal-pleural	10	4.0
Radiological abnormality of grades 0 or 1	150	60.0
Subjects with Normal Vital Capacity	42	16.8
Radiological abnormality of grades 2 or 3		
parenchymal	16	6.4
pleural	18	7.2
parenchymal-pleural	8	3.2

Thomson et al (3) demonstrated that the vital capacity correctly classified all but a few individuals. This finding is most fortunate since the vital capacity is a simply performed, readily available, and inexpensive method of screening for pulmonary functional abnormality in large numbers of workers. It must be emphasized that a decrease in vital capacity in the workers in the study does not necessarily imply that these men have symptoms or are disabled. Indeed, despite a 35 percent incidence of decreased vital capacity, all the workers in the present study were ambulatory, employed, and had few symptoms of significant degree, viz., dyspnea 6 percent and cough 11 percent. Furthermore, the reduction in vital capacity was inconstantly associated with significant physical signs.

Using only the two parameters, vital capacity and FEV_1, the findings in this study confirm the characteristic pattern of interstitial pulmonary disease in asbestosis. As stated previously, 208 (35 percent) had reduced vital capacity. This figure parallels the 30 percent incidence in 56 asbestos workers studied by Kleinfeld et al (12).

The well-known discrepancy between roentgen abnormality and pulmonary function, as seen in asbestosis and other pneumoconioses (12), is again illustrated in this study (Figs. 5 and 6; Table I). There are few data on the incidence of significant radiographic and functional changes correlated not only with each other, but also with duration of exposure. The time course of events, as seen in Figure 5, clearly illustrates that this discrepancy occurs in the early years of exposure. It has been previously emphasized (1, 4, 12) that there is earlier onset of functional, as compared to radiographic abnormality. With longer exposure, the incidence of these abnormalities is comparable. Our own data illustrate quantitatively that the reduction in vital capacity occurs quite early (in more than 25 percent of workers exposed from 5 to 9 years, and in more than 30 percent of those exposed from 10 to 19 years). This contrasts with significant radiological involvement which occurs later (in 23 percent of the group exposed 20 to 29 years). Despite the apparently greater sensitivity of the vital capacity and the parallel incidence of radiographic and functional impairment in workers with protracted exposure, it would be erroneous to believe that all workers with significant radiographic abnormality had functional impairment. In the present study, 16.8 percent of all workers who had significant parenchymal fibrosis by x-ray were without decreased vital capacity.

Summary and Conclusion

(1) 598 ambulatory asbestos workers were screened. In addition to clinical and roentgenographic studies, vital capacity and FEV_1 percent were measured in each subject.

(2) 208 (35 percent) had a reduced vital capacity, and of these, 172 (29 percent) had functional abnormality suggestive of interstitial pulmonary disease (normal FEV_1 percent).

(3) 100 workers (17 percent) had abnormal chest roentgenograms (grades 2 or 3). Parenchymal fibrosis was present in 45, pleural lesions in 37, and both pleural and parenchymal involvement in 18.

(4) Significant symptoms and signs in this ambulatory group of workers were sparse compared to the greater incidence of functional and roentgenographic abnormalities.

(5) Reduction of vital capacity preceded grade 2 or 3 roentgen abnormality by 10 to 15 years. Grade 2 or 3 radiographic changes do not appear until 20 years of exposure. After 30 years of exposure the incidence of functional and radiographic abnormalities is approximately the same.

(6) In 16.8 percent of workers with grade 2 or 3 parenchymal fibrosis there was no decrease in vital capacity.

Acknowledgment

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Rhythmicity of Human Growth Hormone Secretion*

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The principal physiologic suppressor of growth hormone (GH) secretion is glucose, which can suppress basal levels of GH, as well as the secretory response to hypoglycemia, falling blood sugar, exercise, and fasting (1). In contrast, the stimulation of GH secretion by surgical stress (1), bacterial endotoxin administration (2), arginine administration (3), and protein depletion (4) is not completely suppressed by glucose. This differential response suggests that the glucose-suppressible stimuli may be mediated by a shortage of metabolic substrate, whereas the nonsuppressible ones are not.

In addition to the secretion of GH induced by specific exogenous stimuli, plasma GH fluctuates seemingly independently of external stimuli. Quabbe and his co-workers (5) and Hunter and Rigal (6) noted an increase in nocturnal secretion of GH. More recently, several groups of investigators (7–9) have shown that the entry into deep sleep is associated with a burst of GH secretion. The variety of stimuli affecting GH secretion renders difficult the evaluation of the diurnal pattern of hormonal secretion independent of the effects of nutrient provision. We therefore examined the half-hourly pattern of plasma GH levels during several different feeding regimens.

Materials and Methods

Blood was obtained every 30 minutes for 24 hours from a catheter placed in a forearm vein of healthy non-obese volunteer subjects under a variety of experimental conditions, and assayed for glucose (10) and GH (1).

Observation began after an overnight fast; two control specimens were drawn before the start of each regimen. Subjects were confined to bed or chair for the duration of the study, but were permitted to use toilet facilities within 20 feet of the study area.

The following four experimental conditions were studied in four subjects:

FASTING

The subjects were permitted only non-caloric, non-caffeinated beverages.

MEAL FEEDING

The subjects received three liquid formula meals of mixed composition (Metrecal®) containing 17.5 grams of protein, 5.0 grams of fat, and 27.5

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grams of carbohydrate per 8 fluid ounces. Women received a total of 1,800 calories, and men received 2,400 calories. The first meal was ingested after the initial control samples were drawn. The second meal was ingested 4 hours after the first, and the third meal 5 hours after the second.

HALF-HOURLY FEEDING

Subjects received the same number of calories as in the meal feeding experiment of identical liquid formula divided into 21 equicaloric feedings ingested every half-hour for the first 10 hours of the 24 hour observation period.

CONSTANT RATE INTRAVENOUS GLUCOSE INFUSION

Subjects received a continuous intravenous infusion of 15% glucose. During 24 hour infusions, the rate of infusion was 4 cc/minute for men, and 3 cc/minute for women.

During a second set of experiments the following regimens were compared to the constant rate intravenous infusion in two subjects:

INCREASING RATE OF INTRAVENOUS GLUCOSE INFUSION

Subjects received continuous intravenous infusion of 15% glucose/water, initially at 1 cc/minute with a 0.6 cc/minute rate increment every 3 hours, to a final rate of 5.2 cc/minute for the last 3 hours of the 24 hour period.

INTRAVENOUS GLUCOSE AND AMINO ACIDS

Subjects received continuous infusion of 3% Amigen® (a commercially available mixture of amino acids) in 15% glucose/water at 3 cc/minute for 24 hours.

GASTRIC INSTILLATION OF GLUCOSE

Subjects received 15% glucose/water continuously by nasogastric tube at 3 cc/minute for 24 hours.

Results

GLUCOSE

Blood glucose levels were not unusual. Fasting was associated with gradually falling blood glucose levels with little fluctuation (Fig. 1). During meal feedings glucose levels rose minimally after eating and fell shortly thereafter (Fig. 2). During half-hourly feeding, glucose levels remained relatively constant over a narrow range (Fig. 2). Blood glucose levels during glucose infusion were higher than during other regimens and fluctuated considerably despite continuous monitoring of the infusion apparatus (Fig. 2). The administration of glucose intragastrically, the addition of amino acids to the infusion, or the periodic increase in the rate of the glucose infusion produced no consistent alteration (from the constant glucose infusion alone) in the pattern or level of blood glucose concentration (Fig. 3).

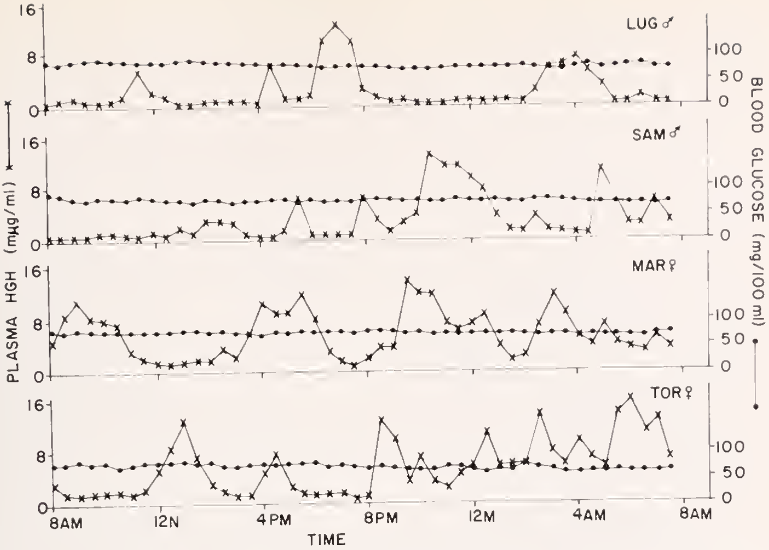

Fig. 1. Plasma HGH and blood glucose during complete fasting in four subjects.

GROWTH HORMONE

Peaks of GH secretion unrelated to blood glucose changes were observed every few hours during fasting (Fig. 1). Meals were followed by a period in which plasma GH levels were initially low but later increased (Fig. 2). During the evening and night following meal ingestion (a period of no further caloric intake), peaks and troughs of plasma GH activity unrelated to specific stimuli were observed (as during fasting). Repetitive nutrient ingestion every 30 minutes suppressed GH levels rapidly and resulted in lower levels of GH during the 10 hours of nibbling than were observed during any other regimen, but small bursts of GH secretion were still observed (Fig. 2), particularly in the women. The 14 hours following the eating period were characterized by multiple peaks of plasma GH. During the glucose infusion experiment, GH levels were uniformly low during the first few hours of infusion but repetitive bursts of GH secretion were observed during the remainder of the 24-hour period (Fig. 2). The GH secretory pattern during the 24-hour glucose infusion did not differ markedly from the secretory pattern during either meal eating or 24 hours of fasting (Fig. 2).

Although GH levels were initially suppressible by glucose or food ingestion, they escaped from glucose suppression after only a few hours during each regimen. An apparently spontaneous, somewhat cyclic, secretion of GH, independent of blood glucose concentration, was observed during fasting, meal feeding, nibbling, and continuous glucose infusion.

The peaks of hormone secretion were also not suppressed by the administration of intravenous glucose at increasing rates, by the administration of glucose plus amino acids, or by the administration of glucose through a naso-

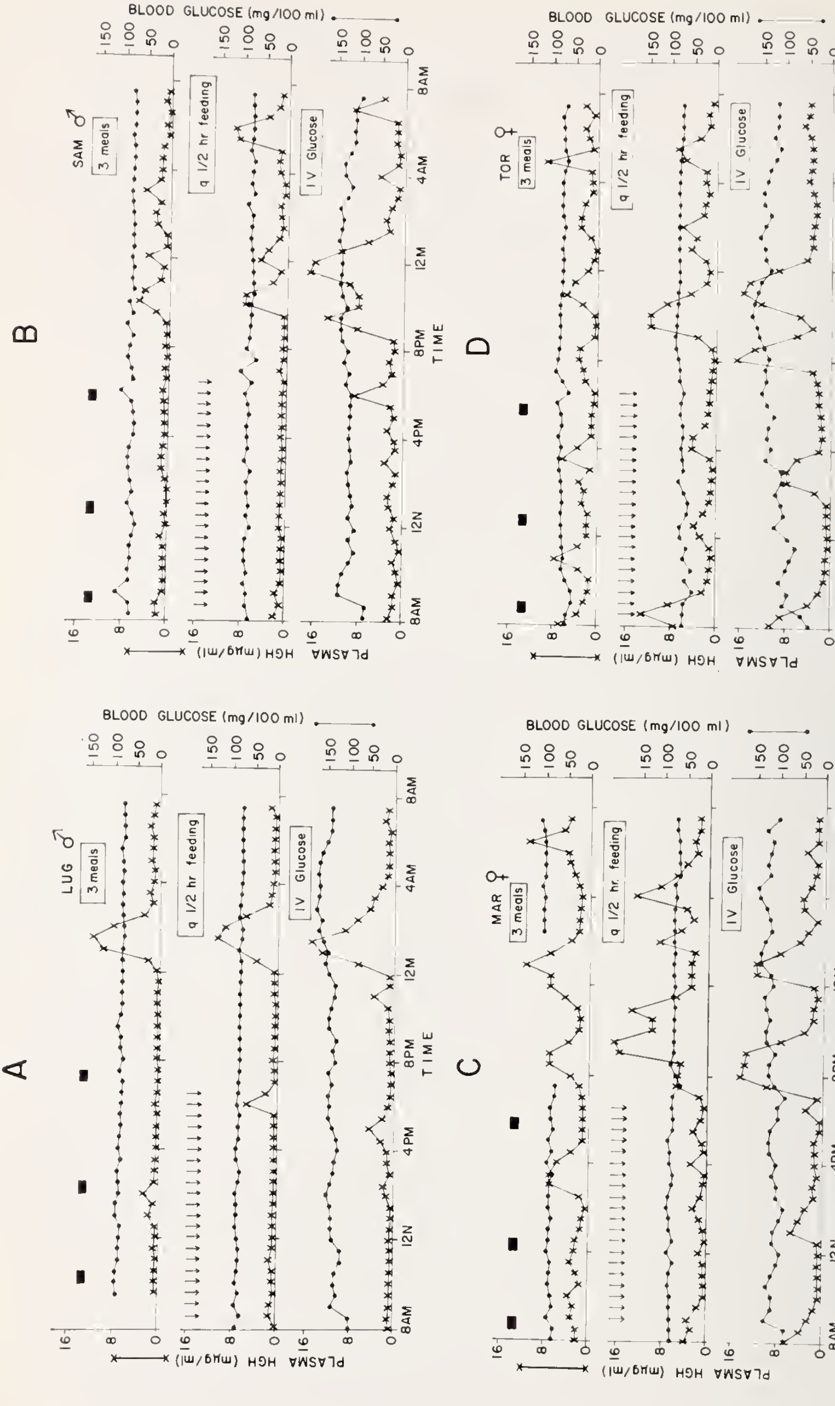

FIG. 2. Plasma HGH and blood glucose in four subjects (A-D) during three experimental regimens. Meals indicated by horizontal bars, half-hourly feedings by vertical arrows.

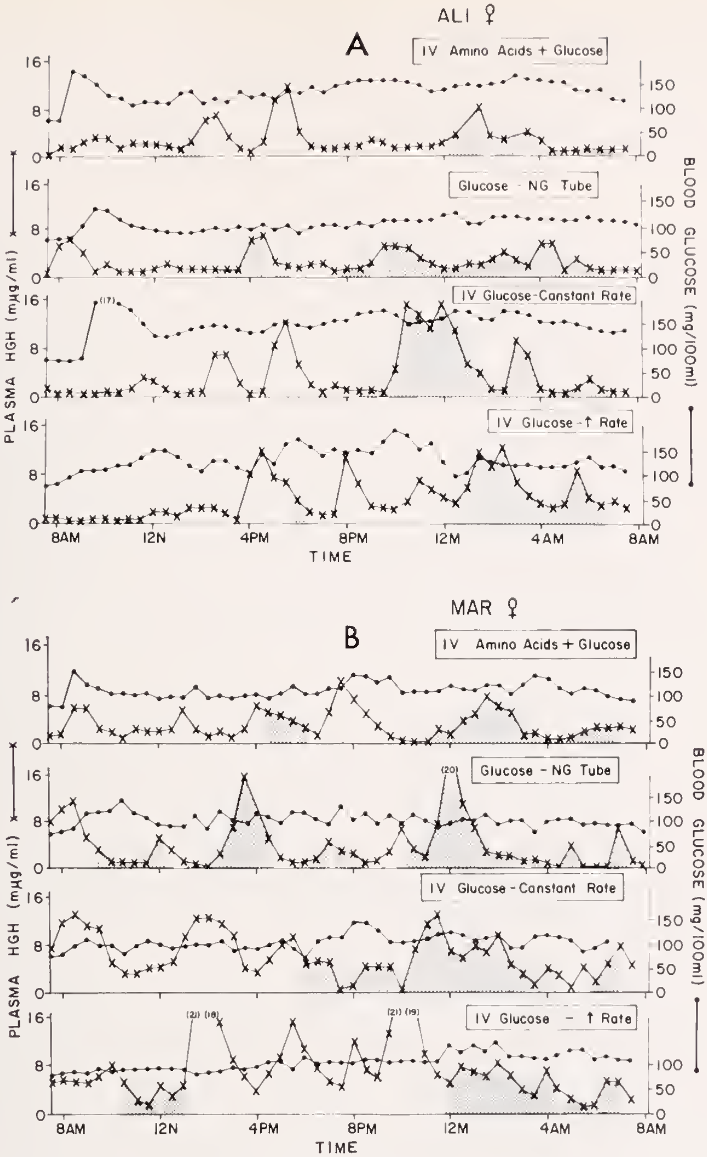

FIG. 3. Plasma HGH and blood glucose in two subjects (A and B) during four experimental regimens. Periods of sleep indicated by shaded areas.

gastric tube. The 24-hour GH secretory pattern under these regimens (Fig. 3) was similar to the patterns described during fasting, either meal schedule, and during glucose infusion. In each instance an initial suppression of GH levels was observed, followed by repetitive elevation of GH levels. During this sequence of experiments, careful note was taken of sleeping patterns, but EEG patterns were not obtained. Although some peaks of GH secretion seemed to coincide with the onset of sleep, others occurred with no obvious relationship to the state of wakefulness (Fig. 3).

All of the subjects were accustomed to the hospital and laboratory environment and manifested no external signs of stress during the experimental observation periods.

Discussion

In the few years since hypoglycemia and carbohydrate deprivation were shown to stimulate GH secretion acutely (11), they have been joined by a host of other stimuli, including surgical stress (1), vasopressin administration (2), pyrogen administration (2), emotional stress (2, 12), arginine administration (3), protein depletion (4), and deep sleep (7–9). The present data describe transient changes in GH levels seemingly unrelated to any of the known stimuli.

Fluctuations in GH levels have been observed previously during prolonged fasting (1, 13), but GH levels returned to normal with feeding or glucose ingestion. In the present experiments, the ingestion of food or of glucose and the infusion of glucose were usually associated with only a short-lived fall in plasma GH levels. Continuous infusion of high doses of glucose was unsuccessful in maintaining plasma GH at low levels for more than a few hours in any of the subjects.

It has previously been observed that whereas acute hypoglycemia stimulates GH secretion, chronic hypoglycemia is not associated with persistently elevated levels of plasma GH (14), presumably because the GH regulatory site accommodates to the continuously low levels of blood glucose. Similarly, accommodation to glucose surfeit might explain the failure of glucose infused at a constant rate to suppress GH secretion; however, periodic increases in the rate of glucose infusion also did not maintain suppression of plasma GH, suggesting that accommodation of the GH regulatory center to glucose administration did not explain the data.

Because higher levels of GH were noted during glucose infusion than during the ingestion of a mixed diet (15), the possibility that protein deprivation might act as a stimulant to GH secretion during the glucose infusion was considered, particularly in view of the elevated GH levels observed in protein depleted children by Pimstone et al (4). The addition of amino acids in physiologic amounts to the glucose infusion did not suppress GH secretion, suggesting that protein deprivation was not a factor, or alternately that the stimulus of protein deprivation is mediated by mechanisms other than total blood levels of amino acids.

To evaluate the possibility that oral feedings might be more effective suppressors of GH secretion than intravenous infusion, glucose was administered through a nasogastric tube at the same rate as during the intravenous infusion experiments. In these studies, too, suppression of GH secretion was not maintained for more than a short time.

While these experiments were in progress, it was shown that the onset of deep sleep was a consistent stimulus to GH secretion (7–9). Unfortunately, detailed sleep observations were not made in our first series of experiments. However, during the later experiments, in which careful note was made of sleep patterns, there were numerous bursts of GH secretion that were unrelated to sleep patterns. Thus, whereas peaks of GH were observed coincident with the onset of deep sleep, these constituted only a fraction of the otherwise unexplained bursts of GH secretion.

The present observations, as well as those in the literature (5, 15), suggest that GH secretion is greatest in the early evening and lowest in the early morning. However, since the various times of day and night are not precisely comparable regarding recency of food ingestion, sleep periodicity, and other variables which influence GH secretion, it is difficult to be certain of a definite intrinsic diurnal pattern of GH secretion.

During almost all the regimens described in the present paper, men had lower overall levels of GH than women. Higher levels in ambulatory women have previously been reported (16, 17), whereas basal levels have been similar in women and men. The present data (15) suggest that even at rest the levels of GH are higher in women than in men throughout most of the day. The explanation for the sex difference is not obvious, but there is now considerable evidence for a peripheral antagonism between estrogens and GH (18–21), suggesting perhaps that the net tissue effect of high levels of GH in the presence of estrogens might be equivalent to low levels of GH in the absence of estrogens.

One subject was studied twice during continuous infusion of high concentrations of glucose (Fig. 4). No consistency of secretory pattern was noted between the 2 experimental days.

The data presented here suggest that a periodic rhythmic secretion of GH occurs independently of all the previously described stimuli to GH secretion. The stimulus for these bursts of secretion is unclear, but the control is likely to be neural, as are the rhythms of other pituitary hormones. Preliminary data suggest that patients with hypothalamic disease have a loss of the peaks of GH secretion (22).

Several seemingly independent mechanisms for GH secretion exist. Multifactorial control of pituitary hormone secretion has been described previously for ACTH, which is controlled by tissue cortisol, by stress, and by a diurnal rhythmicity. So, too, GH may be regulated independently by tissue metabolizable glucose, by stress, by sleep, by adequacy of protein supply, as well as by an intrinsic central nervous system-controlled rhythm, and perhaps by other, as yet undescribed, factors.

Summary

Periodic increases in plasma levels of human growth hormone (HGH) occur in normal subjects, unrelated to any previously described stimuli to HGH secretion. The periodic increases in plasma GH are not completely suppressible by repeated feeding, by continuous oral or intravenous administra-

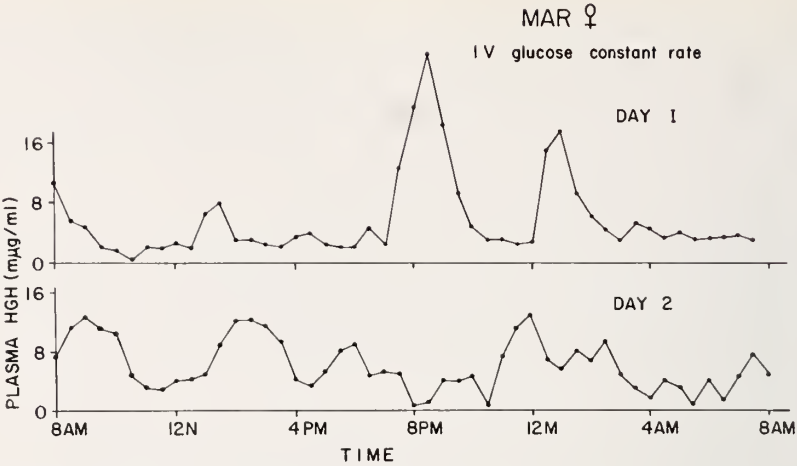

Fig. 4. Plasma HGH on 2 separate experimental days in one subject during continuous glucose infusion.

tion of glucose, or by continuous intravenous infusion of glucose plus amino acids.

Acknowledgments

The assistance of Mr. Jerome Geller and Miss Betty Margul is gratefully acknowledged.

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Skeletal Immaturity, Shortness of Stature, and Delayed Pubescence in a Male with Familial Reciprocal D/D Chromosomal Translocation

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Although the majority of subjects previously reported with a reciprocal translocation of autosomal chromosomes in group 13–15 exhibits a normal phenotype (1–3), a variety of associated congenital clinical manifestations has been observed. Among these abnormalities are mental retardation (3–5), neuromuscular disorders (6, 7), orthopedic deformities (1, 4, 5), oligospermia, azospermia, or sterility (1, 6, 8, 9), primary amenorrhea with lack of development of secondary sexual characteristics (1, 10), and an XXY-type Klinefelter's syndrome (11). A number of subjects with mongolism and chromosomal trisomy 21 were also found to have a D/D translocation (9, 12–17), and one subject developed myelogenous leukemia (18). A 16-year-old male with D/D translocation was reported by Dekaban (3) to have had retarded development since birth, although height and bone age determinations were not included.

Endocrinological investigations were reported by Bühler et al (5) in their studies of a 6½-year-old male with bilateral cryptorchidism, mental retardation, obesity, polydysspondylism, and convergent strabismus, who had underdeveloped external genitalia. The subject was short in stature, with a height of 99 cm. A chromosomal karyotype disclosed a modal number of 45 chromosomes, with a D/D translocation and a normal XY complement. Although the dental age was markedly retarded, bone age was only slightly affected. Plasma growth hormone, thyroid function studies, and the urinary excretion of the 17-ketosteroids and 17-hydroxycorticoids were reportedly normal, although the urinary steroids failed to increase after the intramuscular administration of corticotrophin. Although the subject was prepubertal in appearance, gonadotrophins were detected in the urine. Laparotomy exposed two apparently normal-sized testes, biopsy of one of which revealed histologically an absence of Leydig cells and very few spermatogonia.

The subject described in the present report differs clinically from previously studied cases of D/D translocation by the presence of a considerable retardation in skeletal maturation, together with delayed pubescence and multiple minor congenital anomalies. No associated hormonal deficiency could be demonstrated.

Case Report

E. K., a Caucasian male, was first seen at the age of 17½ years because of shortness of stature. Previous records of height and weight from the age of 7

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years revealed values falling along a growth curve representing two standard deviations below the mean for the corresponding age. The subject's height began to fall below this percentile level at about the age of 11 years, at which time he was 130.2 cm tall. A gradual increase occurred thereafter, the subject attaining a height of 144.8 cm at the age of 15½ years, and a height of 148 cm by 16½ years of age. This height persisted over the course of the following year. Changes in body weight paralleled the progress in height, but had remained stable, between 32 and 33.6 kg, since the age of 14 years.

The subject was born following a normal and uncomplicated full-term pregnancy. No medication or irradiation was administered to the mother during gestation. She was 30 years old, and this was her only pregnancy. Birth weight was 3.5 kg, and length was 53.3 cm. A mild right-sided torticollis was noted congenitally. No cryptorchidism was present, and subsequent genital development was felt to be somewhat retarded. The subject had occasional penile erections, but denied any ejaculations. A high school education was completed in a special class for "slower students," since competition in the regular academic program was unsuccessful. Some sluggishness in activity, as well as in abstract thought, was observed. No hormonal treatment was ever administered.

Although the subject's general health had been good, he contracted rubeola at age 4, varicella at age 6, and mumps at age 7, with no accompanying orchitis. He experienced one apparent grand mal seizure at the age of 5 years, the circumstances surrounding which are not clear. The family history is unremarkable, except for the presence of hypertension. Heights among both parents, both pairs of grandparents, and two maternal uncles varied from 157.5 to 172.7 cm (62 to 68 inches).

Physical examination disclosed a youthful-appearing male, who had a high pitched voice. He had a pulse of 80/minute with a sinus arrhythmia, a blood pressure of 98/55 (right arm) and 104/65 (left arm), a weight of 33.6 kg, a height of 148 cm, and an arm span of 142.2 cm. Crown-to-pubis length was 72.4 cm, and pubis-to-heel length was 75.6 cm. One year later, the height was 150.5 cm, with no change in either arm span or weight. The nasal septum was deviated markedly to the right, and a somewhat high palate was noted. A total of 14 well-developed upper and lower teeth were present. The head exhibited a modest disproportionate elongation in the anterior-posterior diameter. There was faint, fuzzy facial and pubic hair, and no axillary hair evident. The optic fundi were normal. A torticollis was present on the right side. No gynecomastia or breast nodules were palpable. The right scapula was excessively prominent, and a mild pectus carinatum and dorsal dextroscoliosis were observed. The abdomen was slightly protuberant. The penis was somewhat small, measuring approximately 4 cm in length. The urethral orifice was normally situated. The scrotum was faintly pigmented, with some rugal folds. The testes were moderately firm, and about 2.5 cm each in the long axis. A small amount of prostatic tissue was palpable rectally. Skin and hair texture were normal, with multiple pig-

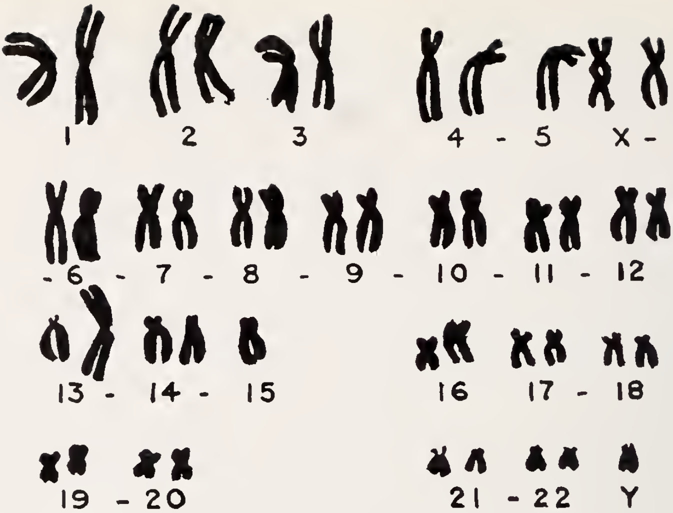

FIG. 1. Chromosomal karyotype of subject E.K., showing translocation involving two chromosomes of the 13-15 group, with a modal number of 45 chromosomes and normal X and Y chromosomes.

mented nevi visible. No acne was present. The remainder of the physical examination was unremarkable.

Laboratory findings included a normal hemogram, erythrocyte sedimentation rate, fasting blood sugar, urea nitrogen, uric acid, calcium, and phosphorus. A VDRL test was negative. Urinalysis was normal. Serum cholesterol was 294 and 246 mg%, alkaline phosphatase was 16.6 and 10.6 KAU, protein-bound iodine was 7.4 μg%, a radioactive triiodothyronine resin uptake was 1.22, and the thyroidal uptake of I^{131} was 32 percent of the administered tracer dose in 24 hours. X-rays of the chest and sella turcica were normal, with no evidence of suprasellar calcifications. X-rays of the hands and wrists, elbows, knees, and pelvis revealed a skeletal age of approximately 12½ to 13 years. There was no epiphysial dysgenesis. Very slight progress was evident on a subsequent skeletal evaluation 9 months later.

The 24-hour urinary excretion of the 17-hydroxycorticoids was 6.8 and 3.6 mg, and of the neutral 17-ketosteroids, 7.2 and 3.3 mg, respectively, on two separate determinations. Four urinary gonadotrophin titers were positive at 10 and negative at 100 mouse uterine units. Of these, one was negative at 20, and another was also positive at 50 mouse uterine units. Skin biopsy revealed a negative sex chromatin pattern. Fasting plasma growth hormone levels were measured by radioimmunoassay (using Wilhelmi HGH standard #705A) on multiple specimens during an insulin tolerance test, during which symptomatic hypoglycemia occurred (lowest blood sugar level was 45 mg%) (19). A maximum growth hormone concentration of 9.4 mμg/ml was observed 2 hours after the intravenous administration of regular insulin 0.1 unit/kg body weight. The control value of plasma growth hormone was 2.4 mμg/ml. This increase was interpreted to represent a normal, although somewhat delayed, response.

A chromosomal karyotype on blood culture disclosed a modal number of 45 chromosomes, with a normal XY sex chromosome complement, but with a translocation involving two chromosomes of the 13–15 group (Fig. 1). This was confirmed by autoradiographic studies. The subject's mother had a similar autosomal translocation, with a normal XX sex chromosome complement, whereas the subject's father had a normal karyotype.

Bilateral testicular biopsy revealed both testes to be immature and prepubertal in histologic appearance. The seminiferous tubules contained only undifferentiated cells and spermatogonia, with an occasional primary spermatocyte. Sertoli cells were present, but no Leydig cells could be identified.

Discussion

Of particular interest in this subject with a D/D chromosomal translocation was the inability to explain the degree of skeletal and testicular immaturity in terms of a deficiency in those hormones known to be associated with bone and genital development. The ability of the anterior pituitary gland to elaborate growth hormone under the stimulus of hypoglycemia was apparently intact, although the constant presence of normal amounts of circulating growth hormone cannot be assured by this means alone. There was no indication of hypothyroidism. Although the delay in the appearance of puberty reflected a functional hypogonadal state, clinical signs of testicular failure and laboratory evidence of severe androgen and gonadotrophin deficiency was lacking. The absence of a eunuchoidal habitus and the presence of genitalia advanced beyond the infantile stage, with scrotal pigmentation, rugal folds, and the occurrence of penile erections, indicated that some androgenic activity existed. The finding on several occasions of normal urinary gonadotrophin excretion attested to the intact functional capability of the adenohypophysis and hypothalamus to secrete their respective stimulating hormones relative to gonadal function. The absence of an elevated urinary gonadotrophin titer, together with a testicular histology devoid of destructive, atrophic, or degenerative changes, precluded the presence of primary hypogonadism. However, the immature appearance of the testes suggested either a period of gonadotrophic stimulation insufficiently prolonged to induce greater development, or an inability of the testes to respond normally to adequate stimulation. Similarly, the epiphysial centers may have been retarded in their development by a delay in the appearance of gonadotrophin secretion, or may have been unable to respond normally to appropriate endogenous hormonal stimulation. The reason for such a lack of skeletal and testicular responsiveness, if it exists, is obscure.

Since so many instances of reciprocal chromosomal D/D translocation are unassociated with obvious clinical abnormalities, it is not possible to determine accurately whether the clinical findings in the subject presented might have been coincidental occurrences. Shortness of stature has not been reported often in this condition, and few evaluations of skeletal development and testicular histology seem to have been performed.

Summary

A 17½-year-old male with a familial reciprocal translocation of the 13–15 group of chromosomes is described. Clinical manifestations included a shortness of stature, with marked retardation of skeletal development, a delayed pubescence, with underdeveloped genitalia and testicular immaturity, and several minor congenital anomalies. Studies of adenohypophysial, gonadal, thyroid, and adrenocortical function disclosed no demonstrable abnormalities. A review of reported subjects with a similar autosomal translocation failed to uncover any other instance of significant skeletal immaturity.

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Studies in Androgen Metabolism[*]

A. LOUIS SOUTHREN, M.D.[†], AND GARY G. GORDON, M.D.

The application of radioisotope techniques to the study of the physiology and pathophysiology of the androgens has provided new information of considerable interest. The results of these studies obtained in our laboratory will be reviewed in detail.

The finding of testosterone in the urine (1, 2) made possible the determination of testosterone production rates (3–5). The validity of the urinary metabolite method for measuring testosterone production, however, was challenged by the observation of Korenman and Lipsett (6, 7) that urinary testosterone glucuronide was not a unique metabolite of plasma testosterone, a portion of the urinary steroid being derived from androstenedione and dehydroisoandrosterone in the periphery (liver) and conjugated before entering the general circulation. As a consequence, testosterone production rates measured by dilution of labelled testosterone overestimated testosterone production to some extent.

To avoid the difficulty with the urinary procedure, testosterone production rates (PR^T) were determined directly in the peripheral circulation from the product of the metabolic clearance rate of testosterone (MCR^T) (vide infra) in plasma following administration of labelled testosterone and the nonisotopic concentration of the steroid (PC^T) as follows: MCR^T (1/day) × PC^T (μg/1) = PR^T (μg/day). This method had the advantage of not requiring the presence of a hypothetical unique metabolite. Southren, Gordon, and Tochimoto (8) reported a mean plasma production rate for 18 normal men and 14 normal women of 6.23 ± 1.73 (SD) and 0.32 ± 10 (SD) mg/day, respectively. Hudson et al (9) reported plasma production rate values for testosterone of 4.6 to 8.8 mg/day (nine subjects) for males and 0.5 to 1.1 mg/day (four subjects) for females. Horton, Shinsato, and Forsham (5) found a mean testosterone production rate in plasma of 6.9 mg/day in five male subjects and 0.8 mg/day in an adult female. Horton and Tait (10) obtained values of 7.8 mg/day (three subjects) and 0.34 mg/day (two subjects) in male and female subjects, respectively. The calculated plasma production rates of testosterone in the male subjects compared reasonably well with the values reported for the urinary metabolite method. However, the female values were considerably less than those obtained by the urinary procedure (3–5). The data indicated that women, in contrast to men, synthesize a larger proportion of their total testosterone in a separate anatomical compartment and conjugate it before entering the general circulation.

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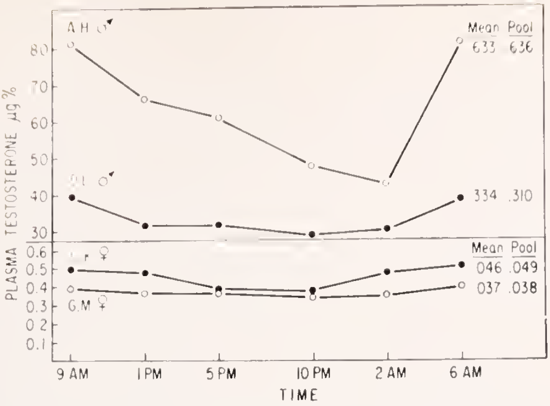

Fig. 1. Pooled (90 minute intervals) and individual (4 hour intervals) plasma testosterone concentrations in normal young men and women during a 24-hour period (from Southren et al (12)).

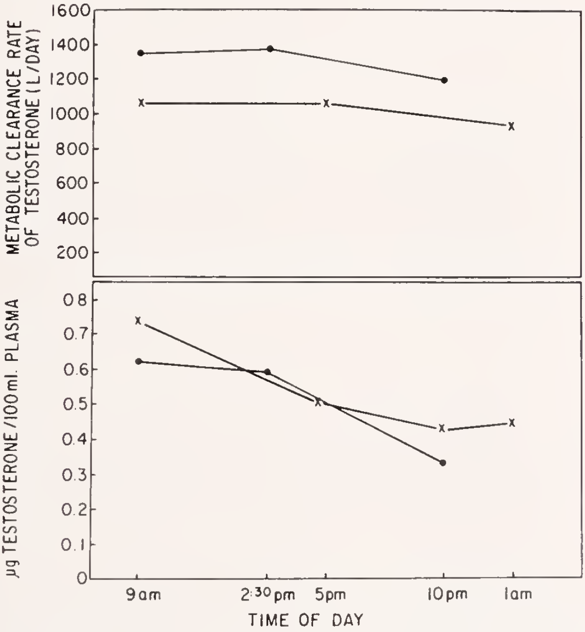

Fig. 2. Metabolic clearance rates and plasma concentrations of testosterone in two normal subjects at various times of the day (from Southren et al (12)).

Diurnal Variation in Plasma Testosterone

Employing a double isotope derivative method (11) for measuring plasma testosterone, plasma levels of the steroid were measured in normal men and women at intervals during a 24 hour period. In one of these studies (12), shown in Figure 1, a distinct diurnal variation was found in the men with the highest concentration of the steroid occurring in the morning. The nadir of the plasma steroid level was between 10 PM and 2 AM. This observation was confirmed by Dray, Reinberg, and Sebaoun (13), Resko and Eik-Nes (14), Lipsett et al (15), Saxena et al (16), and more recently by Crafts et al (17). The use of a single plasma testosterone level to calculate a daily production rate of the steroid introduces a significant error, although it does provide an "instantaneous" plasma production rate. In order to obtain an estimation of the "true" daily plasma production rate, the diurnal variation should be taken into account by obtaining a mean plasma concentration of testosterone from at least three plasma specimens obtained at approximately 8-hour intervals.

The demonstration of a diurnal variation in the concentration of plasma testosterone raised the question as to whether this phenomenon represented an inherent characteristic similar to that seen in many other biological systems, or was related to an alteration in the peripheral metabolism of the steroid. In order to answer this question, the plasma levels of testosterone and the overall rate of metabolism of the steroid (MCR) were measured at intervals during a 24-hour period under basal conditions in the supine position in two normal male subjects (12) (Fig. 2). As can be seen, there was no significant variation found in the values for the MCR's of the steroid during the 24-hour period, while the concomitantly measured plasma concentrations of the hormone decreased sharply. This showed that the observed diurnal variation in plasma testosterone was due to an actual decrease in the production rate of the steroid. Additional evidence for the presence of a diurnal variation in plasma testosterone was obtained by Saxena et al (16), who demonstrated a diurnal variation in plasma FSH and LH. The physiological significance of the diurnal rhythm of plasma testosterone remains to be determined.

Factors Affecting the Metabolic Clearance Rate of Testosterone

The MCR can be defined as the volume of blood irreversibly and completely cleared of steroid per unit time. This is an active process involving both hemodynamic and enzymatic factors. Tait and Burstein (18) have expressed this relationship mathematically as follows: MCR (whole blood) = hepatic blood flow × hepatic extraction + extra hepatic clearance.

SEX

In a study of the MCR^T, by both single injection and constant infusion methods, Southren et al (8, 12, 19) found a highly significant sex difference in the plasma clearance of the steroid, the men having approximately twice the values found in women. Similar results were obtained by Bardin and Lipsett (20). Bardin and Lipsett further showed that the sex difference in MCR^T was not explainable by differences in body surface area, but rather was related to some function of the production rate of the steroid. Our accumulated experience with measurements of the MCR^T's to date have given values for 25 normal men (ages 22 to 30 years) of 1172 ± 240 (SD) 1/day and for 18 normal women (ages 22 to 44 years) of 584 ± 124 (SD) 1/day.

PLASMA CONCENTRATION AND PROTEIN BINDING OF TESTOSTERONE

Tait and Burstein (18) have indicated on a theoretical basis that the MCR of a hormone is independent of its plasma concentration as long as the magnitude of its variation is within physiological limits. In other words, we would not expect a change in the MCR^T within the limits of the diurnal variation of plasma testosterone. Indeed, we have shown this to be true by actual measurement (vida supra). In contrast, changes in the MCR^T can be affected by large scale increases in the plasma concentration of testosterone given both acutely and chronically. Figure 3 shows that orchiectomy, which results in a sharp fall in plasma concentration of testosterone, is associated with a significant decrease in the MCR^T (12). The effect of large increments in the plasma concentration of testosterone on the MCR^T is more complex. Acute *in vivo* increases in the plasma concentration of testosterone under 1.7 μg% in normal women failed to increase the MCR^T (Fig. 4). Increases in the plasma level of the steroid above this value resulted in appreciable increases in the MCR^T. These data suggested that a marked increase in the concentration of unbound steroid in the circulation must be attained in order to influence the MCR^T acutely. This critical level may be related to the capacity of the specific binding protein for testosterone. Consistent with this suggestion are our observations on the effect of increasing the plasma concentration of testosterone on the percentage binding of the steroid *in vitro*. Increasing the plasma concentration of testosterone in normal male and female plasma *in vitro* did not result in a decrease in the percentage binding of the hormone until plasma levels of the steroid far exceeded the male physiologic range (21).

The chronic administration of testosterone consistently resulted in highly

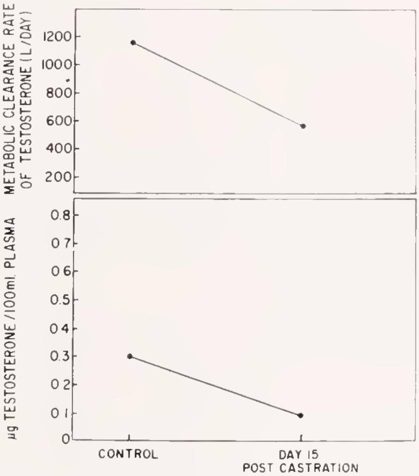

FIG. 3. Metabolic clearance rate and plasma concentration of testosterone in a subject with prostatic carcinoma, before and after bilateral orchiectomy (from Southren et al (12)).

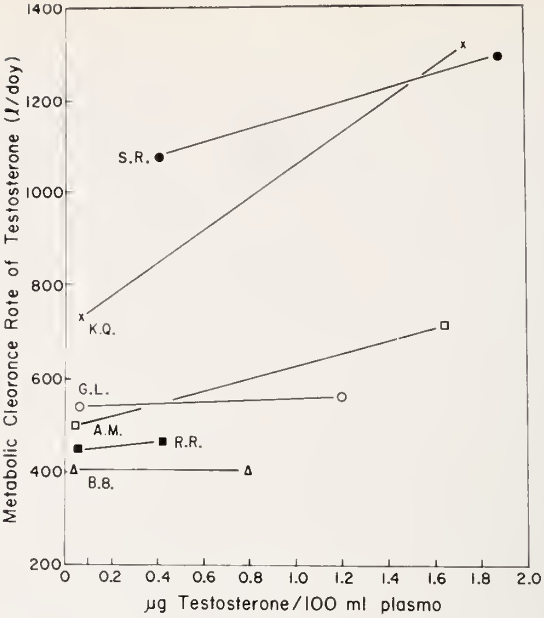

Fig. 4. Effect of acute increases in the plasma concentration of testosterone on the MCR^T in a normal man (S.R.), normal menstruating women (G.L., R.R., A.M., and K.Q.), and in a bilaterally adrenalectomized castrated woman (B.B.) (from Southren et al (8)).

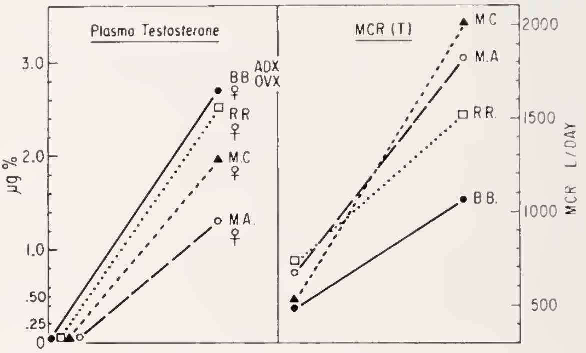

Fig. 5. Effect of two successive weekly intramuscular injections of 200 mg testosterone enanthate on the plasma concentration (PC) and metabolic clearance rate (MCR) of testosterone in three normal women (M.C., M.A., and R.R.) and in an adrenalectomized castrated woman (B.B.) (from Southren et al (12)).

significant increases in the MCR^T, as seen in Figure 5 (12). Since the plasma levels of testosterone attained in these studies exceeded the male physiologic range, it was uncertain as to whether the increment in the MCR^T was due to saturation of the specific testosterone binding protein with a resultant large increase in the concentration of unbound steroid, or whether an additional mechanism was operative, such as induction of enzymes involved in the catabolism of testosterone. In order to investigate these mechanisms, the effect of chronic administration of testosterone on the temporal response of the MCR^T

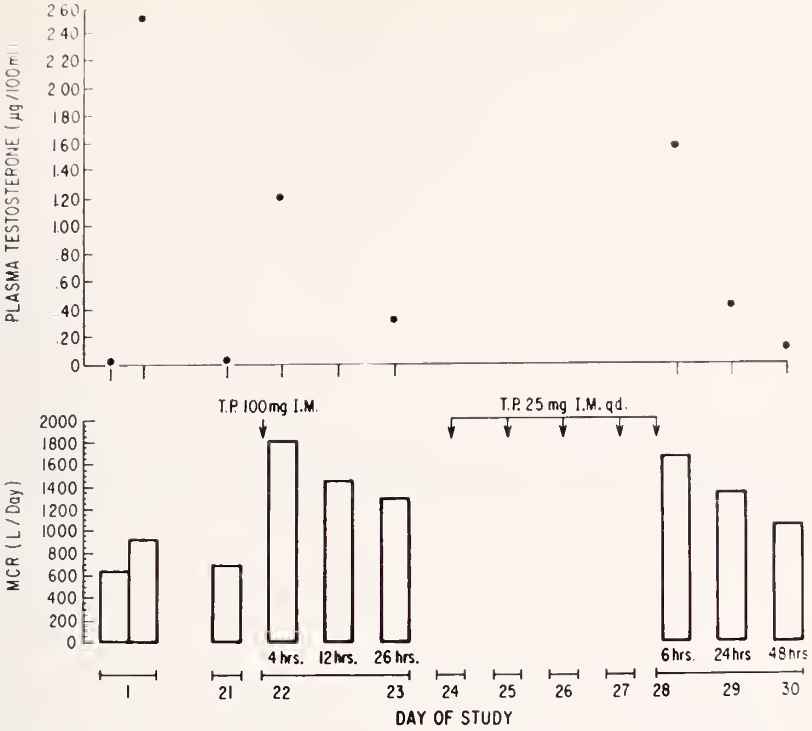

FIG. 6. Effect of acute and chronic increases in the plasma concentration of testosterone on the MCR^T in a man (R.M.) with hypogonadotropic hypogonadism and cirrhosis of the liver. TP = testosterone proprionate (from Southren et al (8)).

was studied serially during and after discontinuation of the steroid in a normal female (21). Increasing the plasma levels of testosterone to the male physiological range and maintaining it for 11 days did not produce a fall in the percentage binding of testosterone until the 5th day of steroid administration. However, continued testosterone administration with maintenance of the plasma level in the male range ultimately produced a fall in the percentage binding of the steroid. These findings suggested that testosterone may produce a decrease in the testosterone binding protein. The measurement of the specific testosterone binding globulin is not feasible at present. However, our demonstration of a decrease in the beta-globulin fraction during testosterone administration raises the possibility of a concomitant fall in the specific testosterone binding fraction of the beta-globulin. Thus, it appeared that testosterone, as well as the estrogens (22, 23), may affect the testosterone binding protein in a fashion similar to their action on the thyroxine binding protein (24).

It is interesting to note that despite a fall in the percentage binding of testosterone to the male range during administration of testosterone to the normal female, the MCR^T's did not increase proportionately as would be expected if protein binding factors were the sole determinant of the metabolic clearance

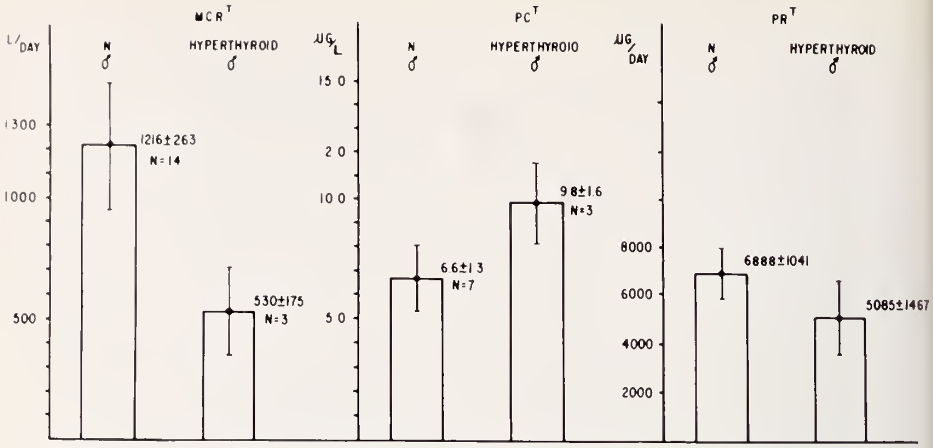

FIG. 7. The mean metabolic clearance rates (MCR^T), plasma concentrations (PC^T) and plasma production rates (PR^T) of testosterone in normal and hyperthyroid males (from Gordon et al (26)).

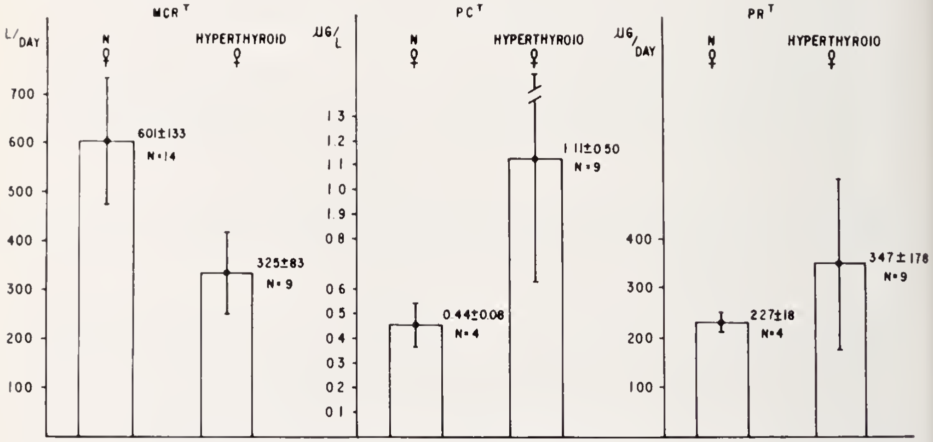

FIG. 8. The mean metabolic clearance rates (MCR^T), plasma concentrations (PC^T), and plasma production rates (PR^T) of testosterone in normal and hyperthyroid females (from Gordon et al (26)).

rate of the steroid. The previously described sex difference in MCR^T (8, 19, 20, 25) has been related to a sex difference in the protein binding of the steroid (22, 23). The present data, however, suggest an additional explanation, namely that the sex difference in MCR^T may be related not only to protein binding factors, but to an effect of chronic exposure to testosterone, possibly at the enzymatic level (8, 21), with induction of testosterone catabolizing enzymes.

Our findings in a hypogonadal male further suggest the importance of enzyme induction in regulating the MCR^T. As can be seen in Figure 6 (8), following both single and serial intramuscular injections of testosterone propionate, the decrease in the plasma levels of the androgen, following the initial

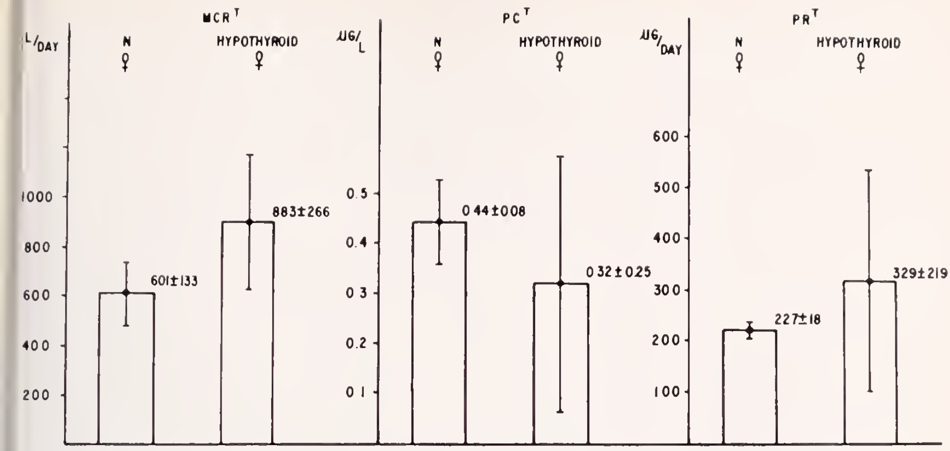

Fig. 9. The mean metabolic clearance rates (MCR^T), plasma concentrations (PC^T), and plasma production rates (PR^T) of testosterone in normal and hypothyroid females (from Gordon et al (26)).

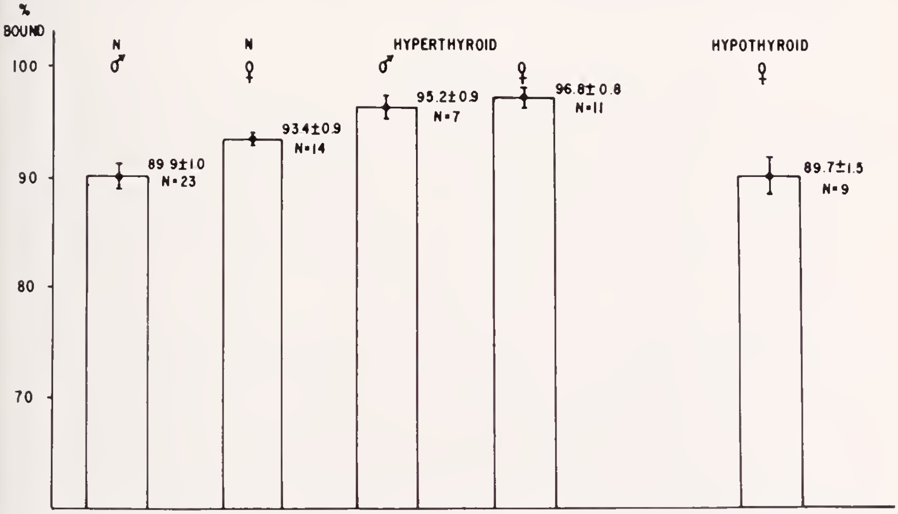

Fig. 10. The percentage binding of testosterone in plasma in normal, hyperthyroid, and hypothyroid subjects (in part from Gordon et al (26)).

increase, was not accompanied by a proportionate fall in the MCR^T as would have been expected if the free level of the steroid were the only critical factor in regulating the MCR^T. Indeed, all of the MCR^T's found during the chronic phases of the study were considerably higher than the MCR^T attained during an acute elevation of plasma testosterone. These data again suggest that induction of testosterone catabolizing enzymes may play an important role in regulating the MCR^T.

We have recently studied androgen metabolism in various pathophysiological states including thyroid dysfunction and cirrhosis of the liver. Disorders of thyroid function were associated with distinct abnormalities in testosterone and androstenedione metabolism (26). The changes in testosterone metabolism are seen in Figures 7 through 10. In hyperthyroidism, there was a significant decrease in both the MCR^T and conversion ratio of testosterone to androstenedione. These changes were associated with an increase in the plasma level of testosterone in both sexes, resulting in a normal plasma production rate of the androgen. The metabolic clearance rate of androstenedione (MCR^A) in hyperthyroidism was not significantly different from normal. However, the conversion ratio of androstenedione to testosterone was appreciably increased. Conversely, in hypothyroidism, there was an increase in both the MCR^T and the conversion ratio of testosterone to androstenedione. Plasma levels of testosterone were low normal, resulting in a normal production rate of the androgen. The effects of hyperthyroidism and hypothyroidism, however, on the kinetic parameters of testosterone metabolism appear paradoxical when contrasted to their effect on the metabolism of cortisol. Peterson (27) reported an increase and decrease, respectively, in the plasma clearance of cortisol in hyperthyroidism and hypothyroidism. In the present study, the opposite effect is noted, in that the MCR^T decreased in hyperthyroidism and increased in hypothyroidism.

One of the mechanisms that may explain the kinetic alterations in hyperthyroidism is a decrease in hepatic blood flow. However, Meyers, Brannon, and Holland (28) have shown a normal to slightly elevated hepatic blood flow in this disorder. A more likely mechanism is a change in the protein binding of testosterone. Indeed, Crépy, Dray, and Sebaoun (29) demonstrated an increase in the protein binding affinity in hyperthyroidism. We (26, 30) studied the percentage binding of testosterone in plasma by an equilibrium dialysis technique and found increased and decreased values, respectively, in hyperthyroidism and hypothyroidism (Fig. 10). In view of our prior observations (8, 12) that the MCR^T is related to the plasma level of the steroid, we would expect the increased serum protein binding affinity for testosterone in hyperthyroidism to be associated with a decreased free (unbound) level of the steroid since the total plasma level of the steroid is elevated, yet the MCR^T is lower than normal. However, a decrease in the free level of testosterone, in the male at least, should produce a compensatory increase in the production of testosterone above the usual rate in an attempt to return the free level of the hormone to normal, as occurs with cortisol during pregnancy or estrogen administration. The fact that this did not occur may indicate an alteration in the regulation of testosterone production. This may be due either to a direct effect of the increased circulating level of thyroid hormone on the pituitary-testicular axis, or indirectly through an alteration in androgen metabolism. Hyperthyroidism is known to increase the relative and absolute amounts of androsterone (a weak androgen) in the urine. The increased production of this metabolite may act to offset the stimulatory effect of a lowered free level of testosterone in this disorder. Evidence in favor of this hypothesis has recently been obtained in our laboratory. Administration of androsterone to rats resulted in a marked decrease in the plasma levels of testosterone. Androsterone thus appears to be active in the feedback regulation of testosterone production in the rat.

In cirrhosis of the liver there was a reduction in the MCR^T and plasma concentration of testosterone resulting in a markedly decreased plasma production rate of the steroid (31). The conversion ratio of testosterone to androstenedione was significantly increased. This observation is consistent with the studies of Breuer, Breuer, and Lisboa (32), who demonstrated an alteration in the pathway of testosterone metabolism toward the 17-oxidoreduction product, androstenedione, at the expense of the A-ring reductase pathway in liver biopsy material from 11 patients with cirrhosis.

The mechanism of the decreased production rate of testosterone in male subjects with cirrhosis may be related to an alteration in estrogen metabolism with resultant secondary hypogonadism. It might be expected, in view of this suggested mechanism, to find an estrogen induced increase in the percentage binding of testosterone. Indeed, these measurements were found to be significantly increased in this disorder. The observation of an increased percentage binding of the steroid in cirrhosis can be attributed to an increased production of testosterone binding protein by the liver in response to the estrogenic stimulus.

Summary

A review of the application of radioisotopes to the *in vivo* study of the kinetics of androgen metabolism, in our laboratory, is presented. The review includes physiologic studies of the diurnal rhythm of plasma testosterone, the sex difference, and other factors affecting the metabolic clearance rate of the androgen and androgen metabolism in various pathophysiologic states.

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Adrenal Cells in Tissue Culture: VI. Some Additional Observations on the Effects of Nucleotides on Steroidogenesis and Glycolysis*

J. KOWAL, M.D.†

Introduction

In recent years an extensive literature has appeared on the possible role of adenosine-3′,5′-monophosphate (cyclic-AMP) as the intracellular intermediate in the action of peptide hormones on their receptor cells (1). The intracellular levels of this nucleotide can be increased by treatment of the target cell with its appropriate hormone, and many of the effects of these hormones can be duplicated by the nucleotide. The ability of β-adrenergic blocking agents, such as propanolol, to block the increases in cyclic-AMP elicited by epinephrine, as well as its metabolic effects, has lent support to this concept (2). In the adrenal cell, cyclic-AMP has been shown to be steroidogenic (3). In addition, ACTH can not only increase the intracellular levels of cyclic-AMP (4), but can also increase the levels of adenyl cyclase activity in membrane-containing fractions of adrenal cells (5).

We have been involved in studies of ACTH action in monolayer cultures of mouse adrenal cortex tumor cells. These cultures provide a viable system for the study of metabolic regulation in the adrenal cell *in vitro*. We (6) have recently reported that cyclic-AMP (1.0mM) added to the medium can stimulate long-term cultures isolated by cloning techniques (7) to the same degree as maximally stimulating levels of ACTH. In contrast to other reports, we found that other adenine nucleotides and nucleosides could stimulate steroidogenesis as well. Guanine, cytosine, and uracil nucleosides and nucleotides had little or no effect. We have recently extended these studies to a new cell line which has properties different from the one previously used. In these cells, high concentrations of cyclic-AMP are only one-third as effective as ACTH, and the other adenine nucleosides and nucleotides are without effect. We report not only our studies of the effects of these compounds on steroidogenesis in this new cell line, but also the effects of several other 3′,5′-cyclic nucleotides on steroidogenesis. Studies with propanolol in these cells have also led to surprising and interesting results, and are also reported herein.

Materials and Methods

The origin of the cells used for these studies, methods of cell culture, and assay of steroid products have been previously reported (8). Nucleosides

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and nucleotides were obtained from California Biochemical Corporation, Sigma Chemical Corporation, and Boehringer-Mannheim Corporation. ^{14}C-glucose was obtained from New England Nuclear Corporation. The method for the determination of ^{14}C-lactate synthesis from ^{14}C-glucose has also been previously reported (9). For these studies, except where indicated, the concentration of the added nucleotide was 1.0mM.

Results and Discussion

Because of the obvious advantages of working with pure cell lines, the clonal cells were utilized for most of the work which had been reported earlier (6). However, because of the possibility that some of the responses to the other adenine nucleotides might have resulted from their adaptation to long-term culture, experiments were also performed with primary cultures of tumors from these cells generated in recipient mice. In virtually every respect, qualitatively similar results were obtained when the clonal cells and the primary cultures were compared. In the past year, we reverted to the use of a different cell line (LCT) obtained as an animal tumor from Dr. Gordon Sato. This cell line mimicks normal adrenal tissue by having a low basal output of steroids and responding to ACTH with a large (eight-to-ten-fold) increase in steroid production. When the same experiments were performed with these cells, the steroidogenic response to cyclic-AMP was substantially lower than that obtained with ACTH (Table I). ATP, ADP, and AMP were not steroidogenic at the same concentration which gave rise to maximum steroidogenic responses in the "responsive" cultures. At the highest concentration of cyclic-AMP employed (3.3mM), the nucleotide was only one-half as effective as ACTH.

There are several possible explanations for the difference in the response of these cell lines to these compounds. The high concentrations of cyclic-AMP required to demonstrate hormone-like effects in most preparations studied has been assumed to be due to the relative impermeability of cell membrane for

TABLE I

Effect of Adenine Nucleotides on Steroidogenesis in Two Cell Lines

	Steroid Output (µg/culture/2 hours)	
	T_{602}	LCT
Control	2.2	0.9
ACTH	6.2	9.2
Cyclic-AMP	7.1	3.2
ATP	7.4	0.8
ADP	6.4	0.7
AMP	6.3	0.7

Effect of adenine nucleotides on steroidogenesis in two cell lines. Each determination represents the average of three Petri dishes (60 × 10mm) of cells incubated for 2 hours in the complete growth medium containing 1.0mM of the appropriate nucleotide. The concentration of ACTH was 10 milliunits/ml. The variation in steroid output was <10 percent.

TABLE II

Comparison of Steroidogenic Activity of C-AMP and DBC-AMP in Two Cell Lines

	Clonal Cells* (μg steroids/2 hours)		LCT (μg steroids/2 hours)	
Control	0.3	0.3	0.7	
ACTH (10 milliunits/culture)	3.5	3.3	10.7	
	C-AMP	DBC-AMP	C-AMP	DBC-AMP
10^{-3}M	3.3	2.0	3.2	7.0
10^{-4}M	1.8	0.7	0.7	1.4
10^{-5}M	1.2	0.3	0.7	0.7
10^{-6}M	0.4	0.3	—	—
10^{-7}M	—	0.3	—	—

* From Kowal and Fiedler (6).

Comparison of steroidogenic activity of cyclic-AMP and dibutyryl-cyclic-AMP in two cell lines. The conditions were the same as described in Table I.

this compound. Another explanation is that the activity of phosphodiesterase, the enzyme which converts cyclic-AMP to AMP, may inactivate most of the material entering the cell. Some increase in sensitivity has been obtained in other cell preparations by using N^6-O′-2-dibutyryl-cyclic-AMP (10). In the "responsive" cells, this compound is less effective than cyclic-AMP (Table II). However, in the cell line with the poor response to cyclic-AMP, dibutyryl-cyclic-AMP is more effective (Table II). At concentrations of 10^{-4}M and 10^{-3}M the dibutyryl derivative elicited greater steroidogenic responses. However, a steroid output comparable to that with optimal levels of ACTH was never obtained.

Further studies are necessary with the "responsive" cells to determine whether the other adenine nucleotides stimulate steroidogenesis through a direct action of their own or raise the intracellular level of cyclic-AMP by increasing the substrate pool. These findings also raise doubts about the significance of using ATP, ADP, or AMP as "controls" in analyzing the specificity of action of cyclic-AMP, since in cells relatively impermeable to cyclic-AMP the other three compounds are less likely to pass through the membrane. Studies currently in progress suggest that the differences in steroidogenic responses to these nucleotides are not related to differences in phosphodiesterase activity.

The recent availability of other cyclic-nucleotides permitted an investigation of the effects of these compounds in both cell lines (Table III). In the "responsive" cells, 2′,3′-cyclic-AMP was an effective as the other adenine nucleotides in stimulating steroid synthesis (Table III). However, it was inert in the "unresponsive" cells. Guanosine-3′,5′-monophosphate, uridine-3′,5′-monophosphate, and inosine-3′,5′-monophosphate were not steroidogenic in either cell line. However, cytidine-3′,5′-cyclic-monophosphate was as effective in stimulating steroid synthesis as cyclic-AMP (Table III). Although

TABLE III

Effect of C-AMP and Other Cyclic Nucleotides in Steroidogenesis from Two Cell Lines

	T$_{602}$ (μg steroids/2 hours	T$_{602}$ (μg steroids/24 hours	LCT (μg steroids/2 hours
Control	1.45	16.1	0.9
ACTH	2.45	24.2	6.3
3′,5′-adenosine monophosphate	3.55	38.4	2.6
2′,3′-adenosine monophosphate	3.10	19.5	1.2
3′,5′-cytidine monophosphate	2.45	38.8	3.6
3′,5′-guanosine monophosphate	—	—	1.1
3′,5′-uridine monophosphate	1.55	19.4	1.2
3′,5′-inosine monophosphate	—	—	0.9

Effect of cyclic-AMP and other cyclic nucleotides on steroidogenesis in two cell lines. Each nucleotide was added to the complete growth medium in a final concentration of 1.0mM.

we have not personally determined the purity of this compound, the manufacturer has provided substantial data on its properties. We are not aware of other reports of hormone-like activity of this compound. It is of interest that both cytosine and adenine have an amino group side chain at the C_6 position on the pyrimidine ring. The possible relationship of this moiety to the specificity of action of these two compounds awaits further studies.

EFFECT OF CYCLIC-AMP AND ATP ON GLYCOLYSIS

Stimulation of steroidogenesis in these cultures with ACTH is generally accompanied by an increase in the rate of utilization of glucose (9). In these cells as well as in other adrenal preparations (11), this has been associated with an increase in lactate production. The effect of cyclic-AMP and ATP on glycolytic activity was studied to determine whether these compounds acted like ACTH on this parameter of adrenal function. Figure 1 describes a typical experiment performed with both types of cultures. In the LCT cells, cyclic-AMP did not significantly increase glycolytic activity. Although it had no effect on steroid output, the addition of ATP to the medium resulted in a diminution in lactate synthesis. Despite this, ACTH added to the cells receiving ATP still effected an increase in glycolysis comparable to the cells receiving ACTH alone. In the "responsive" cell line, the steroidogenic responses to cyclic-AMP and ATP were higher than the response to ACTH. Cyclic-AMP produced the same increase in glycolysis as ACTH; the levels of lactate production in the cells treated with ATP were unchanged from the controls. The dissociation of these effects of the two nucleotides suggests that the mechanism by which ATP stimulates steroidogenesis may not involve an increase in intracellular cyclic-AMP. However, direct proof of this requires measurements of intracellular cyclic-AMP. The ability to dissociate the steroidogenic response and the glycolytic response to ACTH and cyclic-AMP suggests that the increase in steroid output is not triggered by the increase in glycolytic

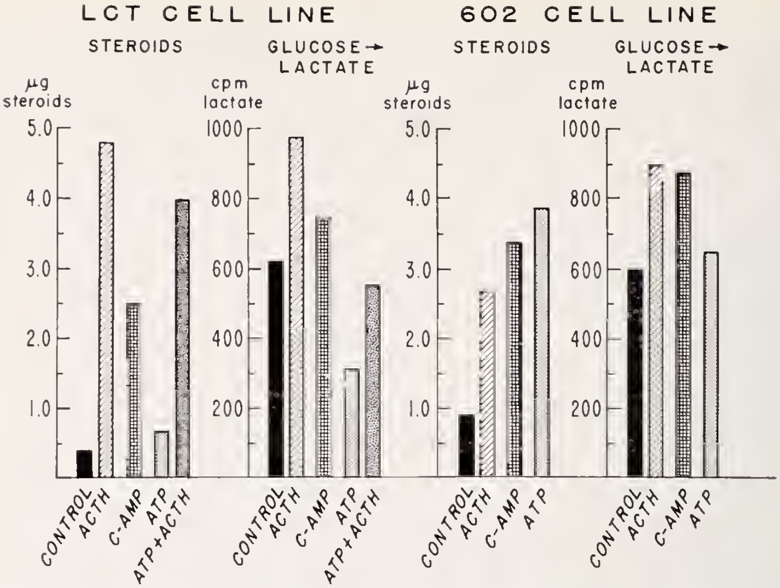

FIG. 1. Effect of ACTH, cyclic-AMP, and ATP on steroid synthesis and glycolysis in two primary culture lines. Three Petri dishes of cells in each group were incubated with ACTH (10 milliunits/culture), cyclic-AMP (1.0mM), or ATP (1.0mM) in the complete medium buffered with Hepes (0.05mM), and supplemented with 0.2μCi ^{14}C-glucose. Incubations were carried out in air for 2 hours. The ordinates represent the quantity of steroids recovered from comparable aliquots of the culture medium, and the radioactivity of the lactate recovered after chromatographic separation (16).

activity. It also appears that measurable glycolytic responses to cyclic-AMP may not occur unless steroidogenic responses comparable to those elicited by ACTH are obtained (see ***Note Added in Proof*** after References).

EFFECT OF PROPANOLOL ON THE STEROIDOGENIC AND GLYCOLYTIC RESPONSE TO ACTH AND CYCLIC-AMP

Propanolol and other β-adrenergic inhibitors have been used to study the effects of epinephrine, glucagon, and ACTH on adenyl cyclase, lipolysis, and glycolysis in fat cells (12, 13). Most of the reported data suggest that propanolol inhibits the increase in adenyl cyclase activity in response to the peptide stimulators, with resultant loss of their metabolic effects. This drug was tested in our cell system to see if it could block the stimulation of steroidogenesis by ACTH. If its primary action was to block the effect of ACTH by inhibiting the stimulation of adenyl cyclase, then the drug should have no effect on the resting rate of steroidogenesis or on the level of steroidogenesis in response to cyclic-AMP. Table IV summarizes the results of an experiment designed to answer this. At a concentration of 100 μg/ml, propanolol decreased the response to ACTH by 50 percent, but contrary to our expectations, not only was the basal level of steriod production decreased by >80 percent, but the response to dibutyryl-cyclic-AMP was also markedly depressed. Thus, propanolol appears to be a potent inhibitor of steroidogenesis. However, in its

TABLE IV

Effect of Propanolol on Steroidogenesis and Glycolysis

	Steroid Output (μg/2 hour/culture)	Lactate Synthesis (μMoles/2 hour/culture)
Control	1.5	2.8
ACTH	12.6	7.0
Propanolol	0.2	6.9
Propanolol + ACTH	5.9	11.2
DBC-AMP	5.8	2.8
Propanolol + DBC-AMP	0.8	6.8

Effect of propanolol on steroidogenesis and glycolysis. LCT cultures were incubated in triplicate in the complete growth medium containing ^{14}C-glucose (200,000 cpm/culture). The concentration of propanolol was 100 μg/ml, and of ACTH 10 milliunits/ml.

TABLE V

Relationship of Concentration of Propanolol to its Effect on Steroidogenesis and Glycolysis

Propanolol (μg/ml Medium)	Steroid Output (μg steroids/culture)		Lactate Synthesis (μMoles/culture)	
	Control	ACTH	Control	ACTH
0	1.2	5.3	2.8	6.7
3.3	1.4	5.3	3.6	6.1
10.0	1.3	6.6	3.6	8.4
33.0	0.3	4.2	4.1	7.6
100.0	0.3	2.0	6.3	8.4

Relationship of concentration of propanolol to its effect on steroidogenesis and glycolysis. Incubations were carried out with LCT cultures for 2 hours in the complete medium containing ^{14}C-glucose (200,000 cpm/culture). The concentration of ACTH was 10 milliunits/ml.

presence, ACTH can still produce substantial increases in steroid output. An additional finding of interest was the effect of propanolol on glycolysis. The drug alone increased glycolysis approximately twofold in the same cultures in which steroidogenesis was inhibited by it. Despite this, in the propanolol inhibited culture, ACTH increased glycolytic activity an additional twofold. Although a good steroidogenic response to dibutyryl-cyclic-AMP could be elicited, the nucleotides had no effect on glycolytic activity. In an additional experiment, the effect of concentrations of propanolol from 1 to 100 μg/ml was investigated. No significant effect was seen at drug concentrations of 10 μg/ml or less (Table V).

These effects of propanolol affirmed the importance of exercising caution in assessing the metabolic effects of a supposedly specific agent. It is evident that the mechanism of its effects in this cell system are independent of any inhibitory action it may have on adenyl cyclase activity. Certain experiments cited in this report raise some questions about the role of cyclic-AMP in stimulatory actions of ACTH other than on steroidogenesis in the adrenal cell, e.g., the ability of propanolol to inhibit the steroidogenic response to dibutyryl-cyclic-AMP more effectively than to ACTH and the dissociation of glycolytic responses to ACTH and dibutyryl-cyclic-AMP in the LCT cells. The use of cell lines with the properties described may provide a novel approach to their answer.

Summary

We have recently reported that monolayer cultures of adrenal cells which respond to ACTH with increased steroid production also had comparable increases in steroidogenesis after treatment with 3′,5′-cyclic-AMP and a variety of other adenine nucleosides and nucleotides (6). In these cells, dibutyryl-cyclic-AMP was less effective than comparable concentrations of cyclic-AMP. A new tumor line has been developed which more closely mimicks normal adrenal cells in its response to ACTH and the adenine nucleotides. In these cells ACTH elicits eight-to-ten-fold increases in steroidogenesis above a low basal output of steroid hormones. Cyclic-AMP (1.0mM) is only one-third as effective as ACTH, and the other adenine nucleotides are without effect. In contrast to the other cells, dibutyryl-cyclic-AMP is more effective than cyclic-AMP, but the stimulation of steroidogenesis by this compound never reaches the levels achieved with ACTH. A number of other 3′,5′ cyclic nucleotides were tested. Of these, only cytosine-3′,5′-monophosphate had steroidogenic activity, the levels of steroid production with this compound reaching those obtained with cyclic-AMP.

The effect of propanolol, a β-adrenergic blocking agent, on steroidogenesis was studied in control, ACTH, and dibutyryl-cyclic-AMP stimulated cells. Steroidogenesis was markedly depressed (>80 percent) in both the controls and dibutyryl-cyclic-AMP cells. ACTH could still stimulate steroidogenesis in the presence of the inhibitor to 50 percent of the levels in the uninhibited cells. Associated with the inhibition of steroidogenesis was a twofold increase in resting glycolytic activity induced by the drug. Despite this, ACTH still increased glycolysis further. The effects of propanolol on steroidogenesis appear to involve a mechanism independent of its known mechanism of action on the adenyl cyclase system. The ability of dibutyryl-cyclic-AMP to stimulate steroidogenesis in the absence of an effect on glycolysis suggests that the effects of ACTH on glycolysis may not be mediated by cyclic-AMP.

Acknowledgments

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Propanolol was obtained from Dr. A. Edwards of Ayerst Laboratories.

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Note added in proof: Additional experiments performed after preparation of this manuscript reveal that full steroidogenic responses can be obtained with cyclic-AMP or cyclic-CMP at a concentration of 10mM. Despite this, little (< 25 percent) or no increase in glycolytic activity occurred in cultures in which ACTH elicited 100 percent increases.

Coexistence of Pheochromocytoma and Carcinoid Syndrome Produced by Metastatic Carcinoid of the Ileum*

R. R. P. WARNER, M.D., AND A. S. BLAUSTEIN, M.D.

Carcinoid tumors with and without the carcinoid syndrome are now well recognized, sometimes associated with the multiple endocrine adenomatosis (pleuriglandular adenomatosis) syndrome (1). This association has predominantly involved carcinoid tumors arising from the bronchi, which are derivatives of the embryonic foregut. No case to our knowledge has hitherto been reported of a midgut carcinoid participating in the pleuriglandular adenomatosis syndrome.

The endocrine manifestations of the tumors associated with carcinoid have varied depending upon the endocrine gland giving origin to the neoplasm. Adenomas arising from the pituitary, parathyroid, adrenal cortex, and pancreatic islets have occurred with carcinoid tumors and have produced the Zollinger-Ellison syndrome, acromegaly, and various other endocrine syndromes (1). A few atypical noncarcinoid tumors of the pancreas with multiple endocrine functions have been described, some producing the carcinoid syndrome (2). In rare instances, the carcinoid syndrome has also been produced by various noncarcinoid tumors originating from organs other than the pancreas (3).

Noteworthy has been the absence of any observation of the concurrence of pheochromocytoma and a carcinoid tumor producing the carcinoid syndrome. Since catecholamines most potently provoke carcinoid flushes and other symptoms of carcinoid syndrome (4, 5), one might predict that a pheochromocytoma in an individual with the carcinoid syndrome would exert an extremely deleterious effect. We are reporting carcinoid syndrome produced by a carcinoid of the ileum with extensive metastases coexisting with a large pheochromocytoma.

Case Report

In April 1966, M.A., a 67-year-old white businessman, first presented for examination by one of us. His complaints of 1 year's duration were progressively worsening diarrhea, facial flushing, and slight weight loss. Many years previously he had been treated for duodenal ulcer but had noted no ulcer symptoms in recent years. Two years previously, because of acute intestinal obstruction, he had undergone ileotransverse colostomy with resection of an

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intussuscepting carcinoid tumor of the ileocecal junction area. Regional lymph nodes were involved by metastases.

On physical examination, this gaunt, purplish-red faced, elderly white male weighed 148 pounds. The blood pressure was 120/80 and the pulse rate was 64/minute and regular. Examination of the heart and lungs revealed no abnormalities. The liver extended for 6½ cm below the right costal margin and was hard, lumpy, and non-tender. Bowel sounds were increased.

Normal values were obtained for complete blood count, fasting blood sugar, blood urea nitrogen, blood electrolytes, total protein, albumin and globulin, bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase, total cholesterol, and prothrombin time. The mean of four blood serum serotonin measurements was 2.45 mcg/ml (normal range 0.08–0.31 mcg/ml). The mean of four assays of the patient's 24 hour urinary excretion of 5-hydroxyindoleacetic acid was 51.4 mg/24 hours (normal range 1.5–7.5 mg/24 hours).

Scan of the liver utilizing Au^{198} disclosed two fairly large voids in the right lobe. These were considered intrahepatic deposits of metastatic carcinoid tumor. On chest x-ray some fine calcifications thought to be in the left lobe of the thyroid were seen. Barium meal radiographic examination of the upper gastrointestinal tract disclosed a deformed duodenal bulb without visible ulcer crater, and very rapid transit of the barium meal through the small intestine.

On June 28, 1966, the patient was first admitted to The Mount Sinai Hospital, where, via percutaneous puncture of the right femoral artery, selective catheterization of the right hepatic artery was performed and 50 mc of $Yttrium^{90}$ was injected into the right hepatic artery. The patient tolerated this well and returned home after 2 days. During the following 2 months liver size decreased moderately without improvement in flushes or diarrhea. During the 3rd month diarrhea and flushing decreased but he noted swelling of the scrotum and right testis with pain in the testis and poorly localized abdominal and right flank pain relieved by bending forward or sitting. Intravenous pyelogram at this time revealed no abnormalities of either the kidneys or ureters except for the right kidney appearing slightly more inferiorly than usual. This was considered to be due to depression of the organ by the massively enlarged liver.

3 mcg of epinephrine was injected intravenously as a provocative test for carcinoid syndrome. Two and one-half minutes after the injection, the blood pressure dropped from 140/80 to 108/60 and a facial flush lasting 1½ minutes occurred. After 1 month an epinephrine provocative injection was given again. A severe facial flush and transient fall in blood pressure were elicited upon the administration of 5 mcg of epinephrine.

Diarrhea and flushing became worse again and were not relieved by a variety of therapeutic agents which included cyproheptadine, SQ 10,643 (Cinnanserin), cyclophosphamide, prednisone, and chlorpromazine. Tincture of opium, methysergide, and phenoxybenzamine given individually and also in combination seemed to reduce somewhat the frequency of the stools but did not alter flushing. Cholestyramine was fairly effective in controlling diarrhea for a period of 2 weeks.

Persisting pain in the right flank, right lower quadrant, and groin appeared to reflect retroperitoneal extension of the carcinoid tumor and was treated by moving strip radiotherapy without relief, but with considerable nausea. On April 28, 1967, the patient was readmitted to The Mount Sinai Hospital because of his intractable pain, edema of legs and scrotum, anorexia, and nausea. At this time a systolic murmur could be heard over the apex of the heart. The electrocardiogram showed only nonspecific T wave changes. Lymphangiography of both legs and abdomen revealed no significant abnormalities. Lidocaine and, subsequently, phenol injection of the right first lumbar spinal nerve completely and permanently relieved the patient's pain. An inferior venacavagram obtained via percutaneous catheterization of the left femoral vein clearly demonstrated a block of the inferior vena cava by a mass in the region of L2 and L3 (Fig. 1). Since this was thought to be due to inoperable metastatic carcinoid, the patient was returned to his home on May 13, 1967. On July 21, 1967, because of continued diarrhea and flushing, treatment with parachlorophenylalanine (PCP) was started. By the time a daily dosage of 3.5 gm was attained, diarrhea was clearly improved, but generalized pruritis occurred. The white blood count at this time was 6,500/mm^3 and the differential count was normal. The pruritis was relieved by stopping the drug for 4 days, but recurred on its resumption. However, the pruritis was then partly controlled by the concurrent administration of cyproheptadine. During the course of PCP treatment, the urinary 5-hydroxyindoleacetic acid excretion fell from pretreatment level of 130 mg/24 hours to a value of 29 mg/24 hours. The serum serotonin showed no decline.

On September 21, 1967, because of rapidly worsening weakness, dyspnea, cough, nausea, and dehydration, the patient was admitted to The Mount Sinai Hospital for the third and final time. PCP treatment was stopped. He was weak, frail, and dehydrated. His face was flushed. The oral temperature was 98.6°F, the pulse rate was 76/minute and regular, and the blood pressure was 140/88. Crepitant rales were present at both lung bases, and cough produced yellowish-grey purulent sputum. No heart murmur was heard. The liver extended for 9 cm below the right costal margin and was firm, lumpy, and nontender. The entire abdominal wall and both lower extremities were markedly edematous.

The hemoglobin was 11.0 gm% and the white blood count was 10,500/mm^3, with a mild shift to the left. Two plus proteinuria was present. Blood electrolytes were normal. Streptococcus viridans was cultured from the sputum. The urinary excretion of 5-hydroxyindoleacetic acid, 1 week after discontinuing PCP, had risen to 134 mg/24 hours, and the serum serotonin was 2.7 mcg/ml. The 24 hour urinary excretion of vanillylmandelic acid (VMA) was 3.5 mcg/mg of creatinine (normal range 0.25–3.50 mcg/mg of creatinine) (6). Chest x-ray showed congestive changes bilaterally, a suggestion of a faint infiltrate at the right lung base, and moderately large bilateral pleural

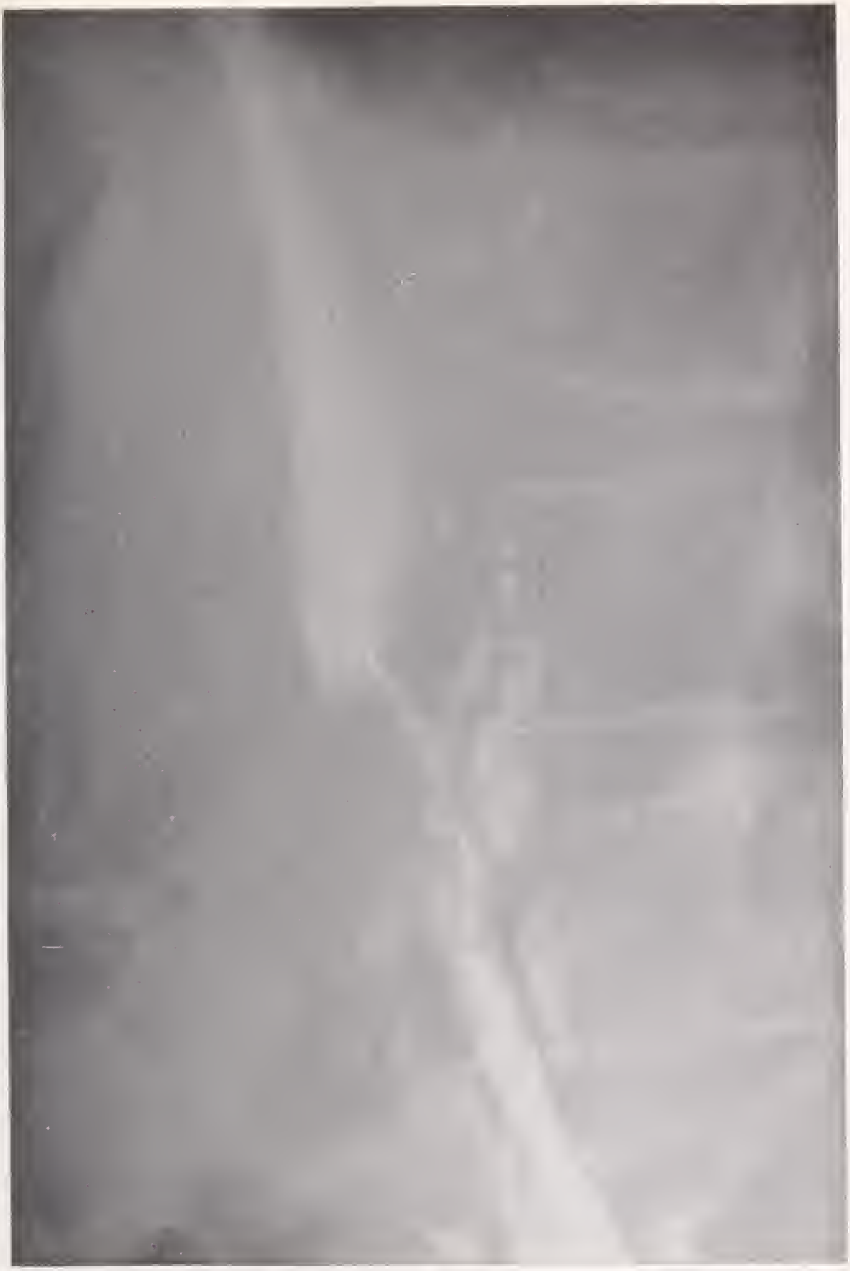

Fig. 1. Marked narrowing of the lumen of the inferior vena cava involving a segment approximately 3 cm long. Contrast material is also seen in collateral vessels, and there is contrast residual from prior lymphangiography in adjacent lymph nodes.

effusions which, on multiple thoracenteses, proved to be transudates. Abdominal paracentesis yielded a small amount of similar fluid. Lung scan showed no evidence of pulmonary infarction. A combination of tincture of opium, methysergide, and phentolamine failed to control flushing or diarrhea and also had no apparent effect on blood pressure. Bilateral quinacrine pleuradesis was carried out and was successful in preventing recurrence of pleural effusions. For 2 weeks the lungs cleared and respiratory symptoms diminished with the additional treatment of antibiotics, oxygen, digitalis, and diuretics. Thereafter, the patient gradually but progressively deteriorated, with recurrent severe flushing and diarrhea, confusion, hypotension, nausea, vomiting, dysphagia, dyspnea, cough, and weakness. He died on November 14, 1967.

Autopsy

GROSS FINDINGS

(1) *Respiratory system:* Pleural effusions and fibrous adhesions to the diaphragm and anterior chest wall were present bilaterally. The lower lobes were fibrotic and had palpable areas of nodularity.

(2) *Cardiovascular system:* The heart was of normal size with mild dilatation of the right ventricle. Two small (2 cm) white tumor metastases were found in the right ventricular myocardium. There was a focal white nodular thickening on the cusps of the pulmonic valve, but the tricuspid, mitral, and aortic valves were normal. The coronary arteries and aorta showed mild atheromatous changes.

(3) *Gastrointestinal system:* Dense adhesions were connected to the bowel and mesentery. The serosa of the small and the large intestine and the mesentery were studded with many small (0.5 cm) white tumor nodules. There was hemorrhagic denudement of the mucosa of the middle third of the esophagus. No gastric or duodenal ulcers were found. Four small plaque-like submucosal nodules (2 cm in diameter) were noted in the ileum.

(4) *Hepatobiliary system:* The liver weighed 1,500 gm and contained many white tumor metastases varying from 0.25 cm to 4 cm in size. Some of the larger tumors were centrally umbilicated, while others were hemorrhagic and necrotic. The gall bladder and bile ducts were normal.

(5) *Endocrine system:* The thyroid gland was enlarged. The right lobe was completely replaced by firm white tumor tissue. The parathyroids, pituitary, and the pancreas were normal. A 6 × 7 cm elliptical adrenal tumor compressed the upper pole of the right kidney (Fig. 2). It was yellow-brown and revealed foci of cystic degeneration and patchy necrosis. The tumor compressed the inferior vena cava, but did not infiltrate its wall. The left adrenal gland was normal.

(6) *Genitourinary system:* The right kidney was distorted by the suprarenal tumor; the left kidney, bladder, and testes were normal. There was a mild degree of nodularity of the prostate.

(7) *Reticuloendothelial system:* Many of the mesenteric, para-pancreatic, and periaortic lymph nodes were enlarged and completely replaced by firm,

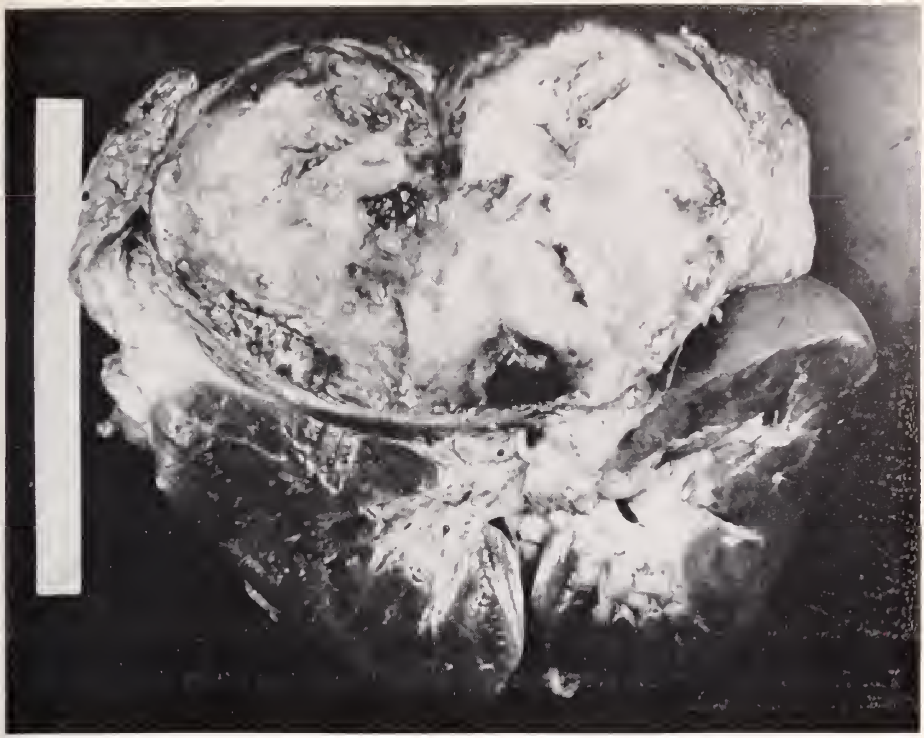

FIG. 2. Cut section of pheochromocytoma and right kidney demonstrating large partially necrotic tumor compressing the upper pole of the kidney.

white tumor. Pulmonic lymph nodes were anthracotic but contained no tumor. The spleen was normal. No tumor metastases were identified in the bone marrow.

The brain, skin, and muscles were unremarkable.

MICROSCOPIC FINDINGS

The carcinoid tumors in the intestine, as well as the numerous metastases, had the classical carcinoid structure. There were nests of regular polygonal cells within a thin connective tissue framework. The cytoplasm was abundant and pale, while the nuclei were vesicular and had prominent nucleoli. There was a mild degree of nuclear atypicality and mitotic figures were infrequent. The serosa and mesentery exhibited marked fibroblastic reaction around the carcinoid nodules. Desmoplasia was also a characteristic of the thyroid metastases.

The pulmonic valve lesion was an avascular, cellular connective tissue with an intact endothelium. An elastic tissue stain of the cusp showed normal elastic fibrils. These findings are diagnostic of carcinoid valve disease (7).

Microscopically, the adrenal tumor was a characteristic pheochromocytoma. There were large cellular trabeculae with mature cells showing nuclear and

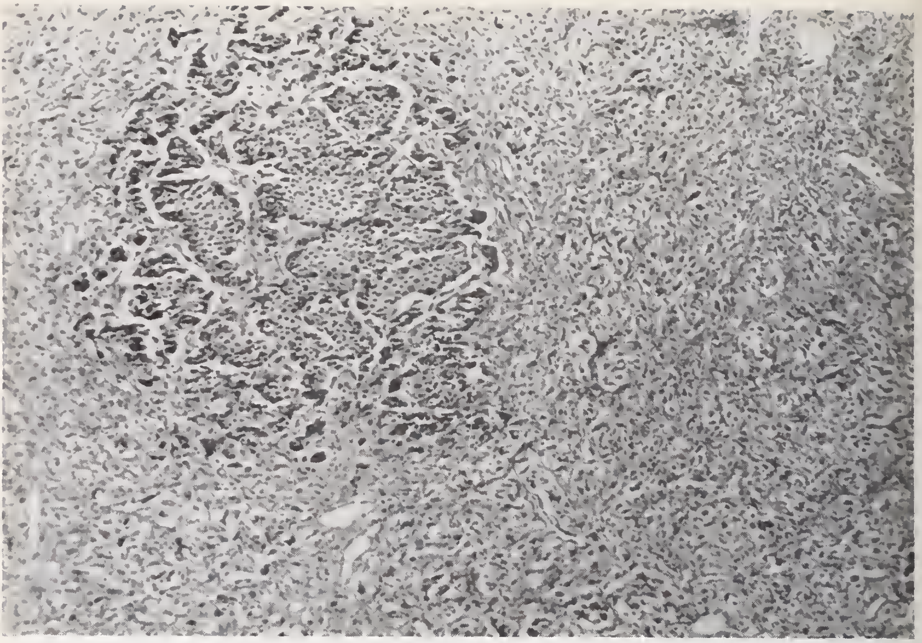

Fig. 3. Microscopic section demonstrating carcinoid metastasis within the pheochromocytoma (H & E × 100).

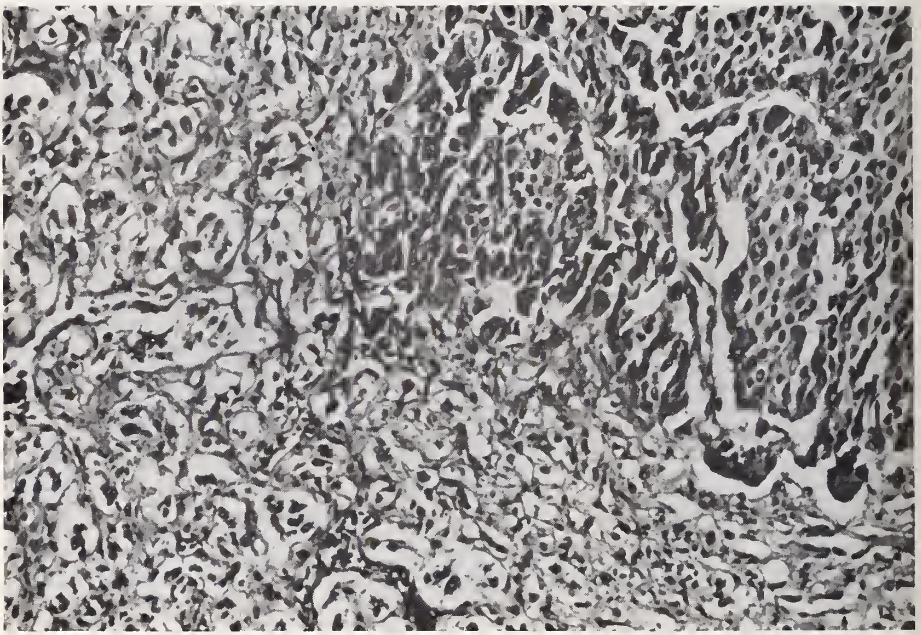

Fig. 4. Higher power view of the carcinoid tumor infiltrating the pheochromocytoma (H & E × 430).

cellular pleomorphism and prominent granular cytoplasm. Many foci of carcinoid were found in the adrenal tumor (Figs. 3 and 4).

Other histologic findings included patchy pulmonary fibrosis with bilateral acute and chronic aspiration pneumonitis. There were two recent pulmonary emboli in the right lung. One small carcinoid focus was found in the pancreas. The kidneys showed mild arterial nephrosclerosis. Adenomatous hyperplasia of the prostate was noted.

Discussion

In recent years, sporadic reports and reviews in the literature have broadened the concept of pleuriglandular adenomatosis (8–10). The association of bilateral pheochromocytomas and medullary thyroid carcinoma (Sipple's syndrome) is well established (11, 12). Four cases are reported in which adrenal medullary tumors and thyroid carcinoma have had concomitant parathyroid adenomas (11, 13). Two of the patients also had clinical signs of hyperparathyroidism (14, 15). Barnard and Jacobson (16) reported a 13-year-old girl with malignant pheochromocytoma who had a small, localized carcinoid of the duodenal papilla at necropsy. This patient did not have the carcinoid syndrome.

Bronchial carcinoids have been reported with associated endocrine disturbances. Goldman (17) was the first to report a patient with a 20-year history of a bronchial lesion who had metastatic bronchial carcinoid and adenomas of the pituitary, both adrenals, and an enlarged pineal at autopsy. Additional scattered reports connected bronchial carcinoids with other endocrinopathies with particular emphasis on acromegaly and Cushing's syndrome. In three such cases recorded by Southren (18), Williams and Celestin (1), and Escovitz and Reingold (19), urinary 5-HIAA excretion was elevated. Of these, only the latter authors' patient was reported to have the carcinoid syndrome. Subsequent to publication, Southren's patient also manifested the clinical features of the carcinoid syndrome (20).

Intestinal carcinoids have rarely been associated with glandular adenomatosis. Gerber and Shields (21) reported a case of a duodenal carcinoid with a non-beta cell tumor of the pancreas. Davis and Kennedy (22) reported a case of gastric carcinoid with metastases and the carcinoid syndrome associated with adrenal hyperplasia. Fisher and Hicks (23) recorded a patient with a duodenal carcinoid, peptic ulcers, and multiple endocrine dysfunction. A rectal carcinoid in a patient with acromegaly was described by Buse, Buse, and Roberts (24). With the exception of Davis and Kennedy's patient, none had the carcinoid syndrome.

The spectrum of carcinoid disease coexisting with, or produced by, other tumors has been further expanded by Moertel and co-workers' report (3) of a patient with medullary thyroid carcinoma, parathyroid adenoma, and carcinoid syndrome with diarrhea, flushing, elevated urinary 5-HIAA, and increased blood 5-HT. No carcinoid tumor was present, and metastatic nodules of the thyroid carcinoma contained markedly increased amounts of

5-HT. Williams (49) has also observed the production of increased amounts of 5-HT by medullary carcinomas of the thyroid. Carcinoid tumors producing the carcinoid syndrome most commonly arise from the distal ileum (25), yet there has been no previously recorded observation of the concurrence of such a midgut lesion with another endocrine tumor. The case we are reporting is apparently the first example of this association.

It is well known that carcinoids arise only where there are specialized epithelial cells of Kulchitsky, the enterochromaffin cells. However, carcinoid tumors arising from the embryonic foregut, midgut, and hindgut may be partially differentiated on the basis of their histological structure, argentaffin and diazo reactions, tumor 5-hydroxytryptamine content and secretion, urinary 5-HIAA, carcinoid syndrome manifestations, and pattern of metastases (26). Therefore, it is reasonable to segregate carcinoid tumors of the bronchus, stomach, duodenum, and pancreas (foregut derivatives) into a distinct group. Though less common than intestinal carcinoids, this group can be recognized as having a greater tendency to be associated with other endocrine tumors.

Our patient had gradual but progressive deterioration with worsening of his carcinoid syndrome and ultimate death over a 2½ year period. Neither sustained nor paroxysmal hypertension were noted at any time during this course. Urinary excretion of VMA was in the upper portion of normal range. This level of excretion of the catecholamine metabolite has been noted by some observers in carcinoid syndrome patients (27, 28), although there have been some such patients reported to have entirely normal excretion of these metabolites (5). Unfortunately, assays of the pheochromocytoma for catecholamine content were not performed. The highly sensitive provocative effect of injection of a minute amount of epinephrine was typical of active carcinoid syndrome (4, 5). All these features we interpret as indicative of the probability that the pheochromocytoma did not significantly contribute to provocation of this patient's carcinoid syndrome. Furthermore, we hypothesize that were the pheochromocytoma truly elaborating increased amount of catecholamines, the patient would have been more tolerant of epinephrine, and hence would not have responded so sensitively to the intravenous injection of a minute amount of this substance. It has been repeatedly observed that there is a great deal of variability from tumor to tumor in the elaboration of catecholamines, with some pheochromocytomas producing large amounts, and others producing very small amounts of these active substances (27, 29). Hypertension has been noted very infrequently in carcinoid syndrome (30), while hypotension, particularly during spontaneous or induced flushes, is characteristic (5, 31).

In spite of the apparently modest catecholamine elaboration by the pheochromocytoma, this tumor probably did contribute to the patient's somewhat accelerated deterioration by obstructing the inferior vena cava. The patient succumbed only 2½ years after the onset of the carcinoid syndrome, whereas patients with a carcinoid syndrome usually survive for longer periods, often for a decade or more (32, 33).

The indolent and persistent recurrence, and the progressive worsening of the patient's diarrhea, flushing, weakness, and general deterioration was a continuous and formidable therapeutic challenge. A varied assortment of drugs was administered, each of which has been reported to be helpful sometimes in the treatment of the carcinoid syndrome. Cyproheptadine (31, 34) and SQ 10,643 (Cinnanserin) (35, 36, 48) (antiserotonins), antitumor therapy with cyclophosphamide (37), prednisone (38, 39) (antibradykinin?), chlorpromazine (40) (antiserotonin, antibradykinin?) (41), and phentolamine (5) (alpha adrenergic blocker) were each without benefit. Tincture of opium, methysergide (42, 43) (antiserotonin), and dibenzyline (5) (alpha adrenergic blocker) helped somewhat in controlling the diarrhea, but not the flushing. The last drug has more often been observed to block flushing rather than diarrhea. Fairly good, though transient, reduction of diarrhea by cholestyramine warrants further trial of this agent for control of the diarrhea of carcinoid syndrome. Pathogenesis of the diarrhea might have been in part due to impaired ileal absorption of bile salts resulting from the combination of rapid transit and the previous resection of a segment of terminal ileum. This could have contributed to the diarrhea by allowing excessive bile acids to reach the colon where they could exert a diarrheogenic effect. As proposed by Hofmann (44), in the presence of a diarrhea-producing mechanism of this type, binding of bile salts in the small intestine by cholestyramine might be expected to ameliorate the diarrhea.

Parachlorophenylalanine, a recently reported highly effective inhibitor of 5-hydroxytryptamine synthesis (45), did prevent diarrhea quite effectively, as had been initially reported in 4 of 5 cases of carcinoid syndrome by Engelman, Lovenberg, and Sjoerdsma (50). However, generalized pruritis, which may have been an allergic reaction to the drug, necessitated withdrawing it (in two other patients treated with PCP by one of us (R.R.P.W.), both developed severe episodes of Loeffler's syndrome, which subsided on withdrawal of the drug, and recurred on subsequent challenge by the drug).

Intraheptic artery injection of Yttrium^{90} has previously been noted to be of palliative benefit when functioning carcinoid tumor is in the liver (46, 47). This treatment afforded some help to our patient which lasted for several months only, and was then overshadowed by symptoms caused by the enlarging pheochromocytoma.

Summary

Carcinoid syndrome produced by carcinoid of the ileum with extensive metastases coexisted with a large, apparently non-functioning pheochromocytoma. To our knowledge, this is the first case of a carcinoid of midgut origin associated with another major endocrine tumor, and also the first reported case of coexistence of the carcinoid syndrome with a pheochromocytoma. Deterioration in the patient's course, which was somewhat more accelerated than usual in the carcinoid syndrome, presented a difficult therapeutic challenge. Many therapeutic modalities were utilized. Some were of limited usefulness.

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Diagnosis and Treatment of Pheochromocytoma*

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Pheochromocytomas are fascinating tumors which are associated with a wide variety of varying signs and symptoms. They produce many different challenging clinical problems and their diagnosis and successful treatment are of great importance since they are associated with a potentially curable form of hypertension. The first antemortem diagnosis of pheochromocytoma was unexpectedly made in 1923 by Masson and Martin (1). Successful removal of the tumor from this patient resulted in profound shock and death. Later, in 1927 and 1929, successful surgical removals of pheochromocytomas were reported (2, 3). The surgical mortality associated with pheochromocytoma, however, continued to be exceptionally high until Grimson et al (4) introduced phentolamine to alleviate the manifestations of the excessive secretion of catecholamines by these tumors. The pharmacologic tests for pheochromocytoma were first introduced in 1945 and were followed by improved surgical management of patients with this tumor (5–10). The introduction of the pharmacologic test for pheochromocytoma greatly enhanced the accuracy and diagnosis of this disease; nevertheless, a relatively high degree of unreliability and intolerable side effects were associated with these procedures (11). In 1957 the diagnosis of pheochromocytoma by means of assay of the urine for the catecholamine metabolites was stimulated by the discovery of 3-methoxy-4-hydroxymandelic acid (VMA) in human urine by Armstrong and McMillan (12). Since that time the definitive diagnosis of pheochromocytoma has been supported by the measurement of the increased excretion of the catecholamine metabolites in the urine. It was subsequently demonstrated by Axelrod and other investigators (13–15) that epinephrine (E) and norepinephrine (NE) are largely metabolized to metanephrine (M) and normetanephrine (NM), respectively, and that these metabolites may be detected accurately in the urine from patients with pheochromocytoma in increased amounts. Weighing the facts that hypertension is a common disease with approximately 20,000,000 cases in the United States, that pheochromocytoma is almost always a fatal tumor when not treated, that the clinical course of pheochromocytoma is frequently identical with that of primary or essential hypertension, and that simple, accurate, and reliable assay techniques are available for the urinary catecholamine metabolites, it is obligatory for the physician to test every patient with hypertensive disease for the presence of pheochromocytoma.

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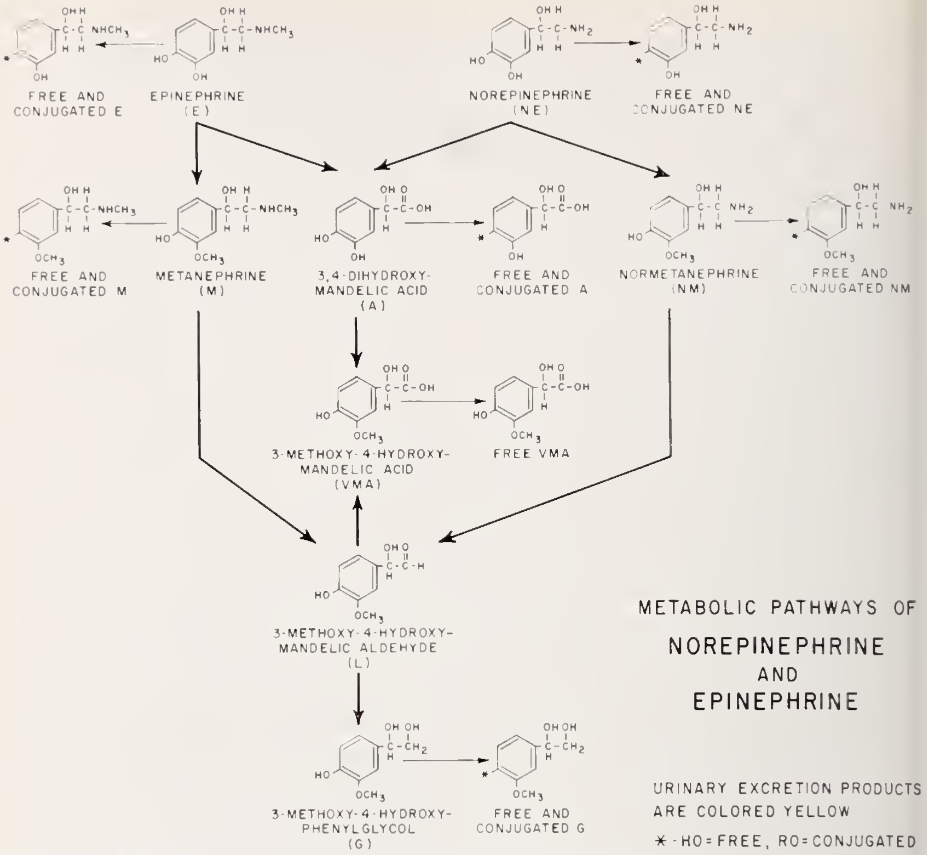
FREE AND CONJUGATED E
EPINEPHRINE (E)
NOREPINEPHRINE (NE)
FREE AND CONJUGATED NE
FREE AND CONJUGATED M
METANEPHRINE (M)
3,4-DIHYDROXY-MANDELIC ACID (A)
FREE AND CONJUGATED A
NORMETANEPHRINE (NM)
FREE AND CONJUGATED NM
3-METHOXY-4-HYDROXY-MANDELIC ACID (VMA)
FREE VMA
3-METHOXY-4-HYDROXY-MANDELIC ALDEHYDE (L)
3-METHOXY-4-HYDROXY-PHENYLGLYCOL (G)
FREE AND CONJUGATED G
METABOLIC PATHWAYS OF
NOREPINEPHRINE
AND
EPINEPHRINE
URINARY EXCRETION PRODUCTS ARE COLORED YELLOW
✱-HO=FREE, RO=CONJUGATED

Fig. 1. Metabolic pathways of NE- and E-degradation indicating the major and alternate routes of metabolism.

Physiopathology

The diagnosis of pheochromocytoma, employing urinary assays for the catecholamine metabolites, was brought into practice in 1957 by the discovery of VMA in human urine (12). Shortly thereafter the diagnostic value of urinary VMA determinations in pheochromocytoma was emphasized by Armstrong employing the two-dimensional paper chromatographic method previously developed by Armstrong, Shaw, and Wall (16). NM, M, and 3-methoxy-4-hydroxyphenylglycol (G) were subsequently shown to be metabolic products of catecholamine metabolism (Fig. 1). The combined amounts of NM and M excreted in the urine of human subjects were shown to equal the amount of VMA excreted and, furthermore, similar quantities of G were also present. The clinical manifestations and hormonal abnormalities in pheochromocytoma are generally related to the increased production of the catecholamines by the tumor.

Assays for the catecholamine metabolites and for the catecholamines have been employed for the diagnosis of pheochromocytoma and, also, to determine the effectiveness and adequacy of surgical removal of the tumor and the presence of multiple tumors or tumor recurrence (15, 17–19). It is frequently difficult or impossible to determine whether a pheochromocytoma is benign or malignant histologically; the presence of apparent metastases at sites distant from the original tumor where no neural crest tissue is found establishes the malignant nature of the neoplasm. Initial reports by Robinson, Smith, and Wittaker of the presence of increased urinary quantitites of homovanillic acid (HVA) and 3-methoxytyramine in a patient with a malignant pheochromocytoma were subsequently followed by other studies indicating that these compounds may be present in increased amounts in the urine of patients with benign tumors (20–23). Although E-producing pheochromocytomas are usually of adrenal origin, frequent exceptions occur (24).

During the past 15 years intensive investigations have been directed toward elucidating the metabolic pathways involved in the synthesis of the catecholamines. The course of catecholamine synthesis involves the conversion of tyrosine to dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase. This reaction, the ring hydroxylation of tyrosine to dopa, is the rate-limiting step in catecholamine synthesis. Aromatic-L-amino acid decarboxylase is the enzyme which catalyzes the conversion of dopa to dihydroxyphenylethylamine (dopamine). Aromatic-L-amino acid decarboxylase inhibitors, such as methyldopa, are clinically available. Dopamine B-oxidase catalyzes the conversion of dopamine to NE, and phenylethanolamine-N-methyltransferase catalyzes the conversion of NE to E. Inhibitors of aromatic-L-amino acid decarboxylase were employed initially to retard the synthesis of the catecholamines in the medical treatment of pheochromocytoma. There was no apparent salutary effect on the symptomatology and course of this disease following the use of these drugs, although the amounts of VMA and tyrosine excreted in the urine were occasionally reduced (25–27). In an attempt to reduce catecholamine synthesis at the rate-limiting first enzymatic step, α-methyltyrosine (αMPT), a tyrosine hydroxylase inhibitor, was administered to patients with pheochromocytoma (25, 28). A greater than 50 percent reduction of catecholamine synthesis was achieved following the administration of αMPT to pheochromocytoma patients. This was correlated with corresponding reductions in the urinary excretions of NM, VMA, and the catecholamines. A general tendency toward blood pressure lowering was also observed. There was, however, no significant blood pressure lowering effect following the administration of αMPT to patients with essential or primary hypertension (25, 28–30).

Manifestations

The clinical manifestations of pheochromocytoma may exhibit a wide variability and appear apparently unrelated since they may have reference to almost any of the organ systems of the body (31, 32). Approximately 1,000 cases of pheochromocytoma have been estimated to occur annually in the United States (33). In over 30 kindred the familial occurrence of pheochromocytoma has been documented; consequently, this disease should be considered in the relatives of all patients with this tumor (34–39). In 1947 Calkins and Howard (39) reported the first case of the familial occurrence of pheochromocytoma; it has been shown, subsequently, that the mode of inheritance is autosomal dominant, with a high degree of penetrance. A higher incidence of multiple tumors, a greater frequency of sustained hypertension, an increased incidence of bilateral medullary carcinoma of the thyroid with amyloid stroma, an absence of the relatively high incidence of right adrenal involvement, and an increased incidence of the neurocutaneous syndromes have been reported in the cases of familial pheochromocytoma (31, 36, 37, 39–41). Pheochromocytoma has been described in all age groups; there is an apparent increased incidence in childhood (42). Pheochromocytoma has also been described in pregnant women (43).

Pheochromocytoma is not associated with body habitus, and the tumor has been reported in both sexes with equal frequency (40, 44–46). Ninety-eight percent of pheochromocytomas are located within the abdominal cavity. A rare pheochromocytoma is found in the neck or within the skull (47–51). The remaining pheochromocytomas are present in the chest cavity. Pheochromocytoma of the right adrenal gland is approximately twice as frequent as pheochromocytoma of the left adrenal gland; 90 percent of all intra-abdominal pheochromocytomas occur in the adrenal gland (42, 52). Multiple pheochromocytomas have been frequently reported; therefore, thorough surgical exploration of the entire abdominal and pelvic cavities should be performed during the surgical treatment of this illness. Although the majority of pheochromocytomas are small, there is, nevertheless, wide variability in the weight and size of these tumors.

The natural history of pheochromocytoma, which is inevitably fatal when not recognized, together with the variability of the clinical manifestations of this disease, dictates that all cases of hypertensive disease be tested for the presence of this potentially curable illness (53). The clinical manifestations of pheochromocytoma may be classified and discussed as follows: paroxysmal hypertension, sustained hypertension, predominantly NE- or E-producing pheochromocytoma, familial pheochromocytoma, the association of pheochromocytoma with other tumors, childhood pheochromocytoma, pheochromocytoma in pregnancy, pheochromocytoma of the urinary bladder, and pheochromocytoma associated with neurocutaneous syndromes. These aspects of the disease will now be examined:

PAROXYSMAL HYPERTENSION

Intermittent hypertension is characteristic of a little more than 50 percent of all patients with pheochromocytoma (31, 42). The hypertensive episodes are frequently associated with intermittent headache, palpitations with or without tachycardia, excessive perspiration, nausea, and pallor. Paroxysmal episodes of trembling or tremor, weakness, anxiety, abdominal pain, chest pain, dyspnea, flushing, numbness or paresthesias, and visual blurring have been noted with decreasing incidence. In any individual patient it is not infrequent that one or more of these symptoms characterize the episode of hypertension. The rate of onset and dissipation of the symptoms is typically brief. Precipitating factors which have been incriminated for these paroxysmal occurrences include sneezing, smoking, posture changes, abdominal pressure, sexual intercourse, and eating. The episodes vary widely in duration and frequency. Half of the episodes are less than 30 minutes in duration. Although the blood pressure and blood glucose concentration are usually normal between these episodes, the urinary excretion of the catecholamine metabolites invariably remains elevated.

SUSTAINED HYPERTENSION

Pheochromocytoma frequently has the clinical appearance of cases of essential or primary hypertension (31). In a little less than half of the cases of pheochromocytoma the hypertension is sustained. Associated with this persistent hypertension there may be evidence of hypermetabolism characterized by tremulousness, fever, excessive perspiration, and weight loss or inability to gain weight. Pheochromocytoma patients who initially have paroxysmal hypertension may subsequently develop sustained hypertension; patients with pheochromocytoma with sustained hypertension may have, moreover, superimposed paroxysmal hypertensive occurrences.

NE- OR E-PRODUCING PHEOCHROMOCYTOMA

When NE is the chief catecholamine secreted by the pheochromocytoma there is a relative paucity of paroxysmal symptoms and hypermetabolic manifestations. In these patients paroxysmal hypotension may be not infrequently observed. The triad of paroxysmal symptoms, shock, and hypotension has been described in these cases.

FAMILIAL PHEOCHROMOCYTOMA

The increased familiar incidence of pheochromocytoma has been described either alone or associated with neurofibromatosis or the von Hipple-Lindau Syndrome (35–38, 54, 55). The familiar occurrence of pheochromocytoma with bilateral tumors, parathyroid adenomas, and bilateral thyroid medullary carcinoma, and the familial occurrence of pheochromocytoma and medullary thyroid carcinoma alone have been emphasized (35, 36, 56, 57).

PHEOCHROMOCYTOMA AND OTHER TUMORS

Thyroid gland carcinoma has been associated with familial and nonfamilial carcinoma. Of all thyroid tumors, medullary carcinoma of the thyroid with amyloid stroma is most frequently associated with pheochromocytoma (37). Patients with thyroid carcinoma associated with bilateral pheochromocytomas have bilateral tumors in approximately 75 percent of the cases. When parathyroid adenoma and pheochromocytoma coexist, thyroid carcinoma is almost always present (34, 58–62). The syndrome of multiple endocrine adenomas has been distinguished from this association (63).

PHEOCHROMOCYTOMA IN CHILDHOOD

A stormy clinical course and multiple and ectopic tumors frequently characterize childhood pheochromocytomas. In childhood these tumors are frequently bilateral (64). Before adolescence there is a male predominance of childhood pheochromocytoma; after puberty no apparent sex association is present. Pheochromocytoma in childhood has been associated with skin mottling, renal artery stenosis, polyuria, polydypsia, and manifestations of obstructive uropathy.

PHEOCHROMOCYTOMA IN PREGNANCY

A disproportionately high mortality and morbidity has been reported in pheochromocytoma associated with pregnancy (65). More than 50 percent of the mothers die, and the infant mortality is almost as high. Pheochromocytoma in pregnancy may mimic toxemia of pregnancy or preeclampsia.

PHEOCHROMOCYTOMA OF THE URINARY BLADDER

Pheochromocytoma involving the urinary bladder wall deserves special comment since it is typically associated with characteristic manifestations. Associated with the act of urination, or shortly thereafter, paroxysmal signs and symptoms suggest the presence of pheochromocytoma of the urinary bladder. In half of the cases hematuria is present together with other symptoms and signs associated with urinary bladder wall tumor. Typically, pheochromocytomas of the urinary bladder wall are small and located intramurally; cystoscopy and cystograms may be normal.

THE NEUROCUTANEOUS SYNDROMES AND PHEOCHROMOCYTOMA

Pheochromocytoma is associated with neurofibromatosis in approximately 10 to 15 percent of the cases. Bilateral pheochromocytomas are not infrequently associated with this combination. Neurofibromatosis, a disease which is predominantly inherited, is associated with an increased incidence of congenital vertebral anomalies, mental retardation, meningiomas, and gliomas. Café au lait spots, generalized hyperpigmentation, and axillary freckling are frequently seen. The association of pheochromocytoma with von Hipple-Lindau Syndrome disease and a combination of both of these illnesses with neurofibromatosis have been noted. Pheochromocytoma may be associated with a wide variety of tumors of the nervous system, including astrocytic tumors of the cerebrum and cerebellum, hemangioblastomas of the spinal cord and cerebellum, paragangliomas, schwannomas, and syringomyelias (56, 57, 66–70).

Differential Diagnosis

Other disorders of catecholamine metabolism occasionally may be confused with pheochromocytoma in children. Increased amounts of catecholamines and their metabolites are produced by neuroblastomas and ganglioneuromas (71–75). Each of these tumors may be associated with hypertension. The characteristic clinical course of neuroblastoma is that of a metastatic malignancy; ganglioneuromas are distinguished by abdominal distention, weight loss, and diarrhea. Pheochromocytoma may mimic subjects with Riley-Day Syndrome (familial dysautonomia), a congenital disease of children in which impaired temperature regulation, hypertension, defective lacrimation, indifference to pain, postural hypotension, nausea, sweating, hyporeflexia, and skin lesions are the cardinal manifestations (76). Acrodynia is occasionally confused with pheochromocytoma. Acrodynia is a disease of infancy and childhood which is associated with hypertension, sweating, and tachycardia. Increased urinary excretion of the catecholamines has been observed in these patients (77).

A wide variety of diseases may be confused with the paroxysmal symptoms of pheochromocytoma, and these must be carefully distinguished by the attending physician. Acute anxiety states, migraine headaches, and vasodilating headaches may be associated with many of the intermittent manifestations of pheochromocytoma. Diencephalic autonomic epileptic episodes are not an infrequent source of confusion. Pheochromocytoma may be mistaken for hypoglycemia episodes, coronary insufficiency, and even the menopausal syndrome.

Diagnosis

The pharmacologic tests for pheochromocytoma were described in 1945 (5). They measure either the hypotensive response to adrenergic blocking drugs or the hypertensive reaction to certain provocative agents. The information obtained from these pharmacologic testing procedures is significantly less than the specific information obtained from the urinary assays for the catecholamines and their metabolites in establishing the diagnosis of this tumor. These tests are seldom indicated at present not only for this reason, but also for the high incidence of associated serious complications.

Only a few of the large number of pharmacologic tests for pheochromocytoma that have been described are currently and occasionally employed (78). Assays for the catecholamines and the catecholamine metabolites in the urine or blood, together with the patient's pharmacologic and physiologic response, may be simultaneously made during the provocative tests. In approximately 20 to 25 percent of the cases the histamine provocative tests may give a false negative result. Significant morbidity and mortality have been reported, moreover, in association with histamine testing (79, 80).

Engelman and Sjoerdsma described the details of a provocative test for pheochromocytoma employing the pressor responsiveness to tyramine in 1964 (81–86). In 20 to 25 percent of the cases false negative responses have been reported with the tyramine pharmacologic test; false positive results in less than 5 percent of the cases (85). The most frequent and significant side effect is palpitation, and there is no correlation between tyramine responsiveness and the urinary excretions of the catecholamine metabolites and the catecholamines. It has been reported that the sensitivity of the tyramine provocative test is less than that of the cold pressor-histamine test (82, 87).

Lawrence (88) introduced a glucagon provocative test for pheochromocytoma in 1967. Although wide experience has not been accumulated with this new test, serious side effects have not yet been reported.

In 1951 Emlet et al introduced the phentolamine pharmacologic test for pheochromocytoma (9, 89). Of all of the pharmacologic tests for pheochromocytoma which measure a hypotensive response in patients with sustained hypertension, this is probably the safest. Occasionally, deaths from side effects, however, have been reported (90, 91). At the present time the pharmacologic tests for pheochromocytoma are rarely indicated since reliable assay procedures are available to establish the diagnosis safely and accurately by means of measurement of the concentrations of the catecholamines and their metabolites in body fluids.

The diagnosis of pheochromocytoma is established with a high degree of reliability by assay procedures for the catecholamines and their metabolites in the urine or plasma. Although a large number of different tests have been devised for these substances, analysis of the urine for the increased excretions of the metanephrines, either in combination or separately, is the test procedure which offers the highest degree of accuracy associated with simplicity and rapidity of analysis. All of these tests are based upon the increased urinary excretion and an increased plasma level of the catecholamines and their metabolites in patients with pheochromocytoma (Fig. 1). Although several assay techniques are available for the measurement of the urinary excretion of the catecholamines, one of these tests has important advantages over all of the others (92–97). This is a new, specific, and sensitive double isotope derivative method for total catecholamines in urine and plasma (97). Hypoglycemia, emotional stress, exogenous catecholamines in food, and the administration of nose drops and various drugs in patients with uremia and high cerebral spinal fluid pressure have been reported to give false positive determinations with many of these catecholamine assays. Furthermore, there is a wide range of normal values for all of these tests, which may obscure a pheochromocytoma which is minimally secreting catecholamines. Numerous technical differences and length of procedure, moreover, are additional drawbacks (98).

Plasma catecholamines may be assayed with various techniques (92, 93, 97–103). Elevated plasma catecholamine levels have been described in patients with various tumors, and false positive plasma catecholamine values have been observed in circumstances similar to those which are associated with false positive excretion values (104–106). No false positive results have been reported thus far with the double isotope derivative method for assay of the catecholamines in biological specimens; besides, application of this method to human plasma samples obtained at vena cava catheterization has accurately localized an ectopic pheochromocytoma (97). Because of the fact that urinary assays for the catecholamine metabolites are highly reliable and simple to perform, together with the known technical differences inherent in the plasma assays for the catecholamines, it appears that catecholamine assays will be used infrequently.

Three catecholamine metabolites, of all of the catecholamine metabolites which are present in the urine in increased amounts in patients with pheochromocytoma, have been utilized with great frequency in order to establish the diagnosis of this disease. Of the three catecholamine metabolites, VMA, NM, and M, assays for NM and M are generally recognized to have particular advantages over measurements for VMA. Several urinary assays for the measurement of VMA have been reported (72, 95, 107–112). Dietary factors, shock, the ingestion of certain drugs, coronary insufficiency, and carcinoma have been reported to give rise to false positive results. Monoamine oxidase inhibitors and decarboxylase inhibitors may result in false low values. Utilizing any of the tests which analyze for the urinary excretion of VMA in order to establish the diagnosis of pheochromocytoma, the incidence of false positive results is approximately 10 to 15 percent (83, 113, 114). For this reason, almost all hypertensive research laboratories and clinics prefer and advocate an assay procedure for the urinary metanephrines in order to establish the diagnosis of pheochromocytoma (82, 113–115, 118).

The simplest and most suitable assay technique for the diagnosis of pheochromocytoma now existing is analysis of the urine for the combined metanephrines or for separate NM and M. An assortment of assay techniques are available involving either periodate oxidation coupled with spectrophotometric measurement, or electrophoresis with or without chromatography (95, 113, 114, 118–125, 127). Generally, these urinary measurements for the metanephrines are easier to perform and more reliable than assay techniques for other catecholamine metabolites. We have had personal knowledge with over 1,000 urine specimens in our laboratory, and have never encountered either a false positive or a false negative result in the diagnosis of pheochromocytoma with a chromatoelectrophoretic technique which assays for the urinary combined metanephrines and G (113, 118). Concentrations of the combined metanephrines and separate NM and M may be reduced in the urine after the administration of certain pharmacologic agents (128). An occasional patient with pheochromocytoma may have increased urinary excretion values for either NM or M associated with normal or low values for the other methylated metabolite. In these cases, however, the combined urinary M value is, nevertheless, elevated (124). In order to establish the diagnosis of pheochromocytoma, urinary assay for the combined metanephrines is the preferred test.

Patients with pheochromocytoma have an increased secretion of NE, as measured by the apparent NE secretion rate test (ANESR) (124, 125, 127–132). In this test, patients with pheochromocytoma have an increased urinary excretion of non-radioactive NM and an increased urinary excretion of normetanephrine-H^3 (NM–H^3) following the administration of a trace dose of Dl-norepinephrine-7-H^3 in the subsequent 24-hour urine collection. The specific activity of the normetanephrine in the urine is decreased, and the ANESR, which is calculated in the usual fashion, is increased. Contrariwise, patients with essential hypertension have normal values for urinary non-radioactive NM, increased values for urinary NM-H^3, increased values for NM specific activity in the urine, and decreased ANESR.

Complications

Complications of pheochromocytoma are not unlike those which are associated with essential or primary hypertension. Cerebrovascular accidents, retinopathy, diffuse vascular disease, malignant hypertension, and nephrosclerosis with renal insufficiency have been reported (31, 42, 53, 133, 134). Many cardiac complications of pheochromocytoma have been reported. Patients with pheochromocytoma may develop a focal myocarditis (135). Congestive heart failure and pulmonary edema may complicate the myocarditis. Discrete areas of myocardial necrosis associated with an inflammatory cell reaction usually occur around the small blood vessels of the myocardium. Myocardial infarction, cardiac arrhythmias, and coronary artery arteriosclerosis are habitually present in these patients (136–138). Shock and hypotension may complicate the tachy-arrhythmias (136). The presence of ventricular tachycardia and ventricular fibrillation has been stressed, although all of the various tachy-arrhythmias have been described in this disease (136).

Pheochromocytoma appears to be associated with an increased incidence of cholelithiasis (49, 115). Primary manifestations of pheochromocytoma may resemble diabetes mellitus, with hyperglycemia, glycosuria, and, occasionally, ketoacidosis (139). Surgical removal of the pheochromocytoma ameliorates, either partially or completely, the impaired glucose tolerance seen in this disease (140, 141). Inhibition of insulin release and resistance to the hypoglycemic action of insulin have been incriminated in the abnormal carbohydrate metabolism coexistent with pheochromocytoma (139, 142). Pheochromocytoma and diabetes mellitus may coexist in the same patient; diabetic complications are generally enhanced in these patients.

Further Diagnostic Procedures

Since it is commonly agreed that over 98 percent of all pheochromocytomas are located between the diaphragm and the pelvic floor, and that multiple pheochromocytomas are present in approximately 20 percent of the patients with this tumor, there is an indisputed inaccuracy of tumor localization by roentgen procedures. For this reason it is recommended that additional diagnostic procedures be restricted to a minimum (31, 115–117). X-rays of the chest and skull set aside the possibility for pheochromocytomas in these locations. An intravenous pyelogram with laminography of the renal areas has been performed frequently without excessive mortality or morbidity; this procedure provides important information concerning renal function and, occasionally, tumor localization. Cholecystography may reveal the presence of cholelithiasis; the diseased gallbladder may be electively eliminated at the time of the abdominal surgery. Pneumography and arteriography and other forms of radiography involving manipulative procedures have been associated with significant mortality and morbidity (115, 143).

Management and Treatment

MEDICAL

The treatment of choice for pheochromocytoma is surgical removal of the tumor. Nine out of ten patients with pheochromocytoma are surgical candidates. Patients who are not surgical candidates include those with metastatic malignant pheochromocytoma, and patients with benign pheochromocytoma who would not survive the surgical procedure. The medical management of pheocromocytoma includes the preoperative preparation of patients and the chronic medical treatment of patients who are not surgical candidates. Alpha-adrenergic blocking drugs are usually administered preoperatively to hypertensive patients with pheochromocytoma. Phentolamine and phenoxybenzamine are the two currently available therapeutically efficacious α-adrenergic blocking agents. Phenoxybenzamine, which may be administered orally in single or divided doses, has advantages over phentolamine, which must be parenterally administered. Prevention of hypertensive episodes, blood pressure reduction, and amelioration of many of the manifestations of hypermetabolism may be obtained. Phenoxybenzamine has been used in the chronic treatment of patients with either benign or malignant pheochromocytoma (26, 115, 144, 145). The tyrosine hydroxylase inhibitor, αMPT, has been employed in patients with pheochromocytoma to decrease the excessive catecholamine secretion (25, 28, 146). General clinical improvement and significant blood pressure reduction have been obtained with this drug. It is not unlikely that either this drug or an analogue will be used with increased frequency in the medical management of patients with pheochromocytoma.

Most patients with pheochromocytoma have normal plasma volumes and total blood volumes; an occasional patient has reduced values for these measurements (115). It has been established by clinical observation that blood replacement at the time of operation or repletion of the blood volume in excess of the amount lost is of paramount importance in the reversal of the hypotension that frequently occurs after tumor resection (147). Pheochromocytoma may rarely be associated with the increased secretion of an erythropoiesis-stimulating factor and erythrocytosis (158). Careful continued measurements of the arterial blood pressure, central venous pressure, electrocardiogram, and heart rate should be performed in all patients with pheochromocytoma during surgery. The anesthetic of choice is halothane. The increased cardiac excitability caused by E is effectively reversed by the antiarrhythmic drug, lidocaine (149). Propranolol, a beta-adrenergic blocking drug, is even more effective than lidocaine in the control of catecholamine-induced arrhythmia (150).

SURGICAL

All patients with intra-abdominal pheochromocytoma should have a thorough abdominal exploration, recognizing the fact that many of these tumors are multiple (115, 151–153). A transabdominal surgical approach is required to expose the entire abdominal cavity adequately. Particular attention should be directed toward all of the abdominal areas of chromaffin tissue localization, including the adrenal glands, necrotic tissue, pelvic organs, and organs of Zuckerkandl at the bifurcation of the aorta. Palpation of suspicious lesions and measurements of the arterial blood pressure may add to the localization of these tumors. In order to eliminate the abdominal-tumor bearing tissue, it has been advocated that the adjacent fat and fibrous tissue in the neighborhood of the adrenal gland be removed together with the pheochromocytoma of that particular gland. Malignant pheochromocytoma may not have characteristic histologic features; therefore, all specimens should be thoroughly examined in the operating room for evidence of malignancy characterized by local tissue invasiveness (154). If malignancy is established, then the local lymph nodes should be removed. Blood pressure reduction from hypertensive to normotensive levels following isolation of the adrenal blood vessels with occlusion of the venous drainage implies that all of the disease has been discovered and identified. An internist should be on the operating floor at the time of surgery to assist in the administration of the appropriate drugs.

Conclusion

Distinct and significant advances have been achieved in the diagnosis and treatment of pheochromocytoma in recent years. It is not surprising that this has been accomplished by the intermixing of many medical disciplines that have applied individual aptitude, abilities, techniques, and special endowment to this complex disease.

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Early History of the U. S. Public Health Service (Marine) Hospitals: The First Federal Prepaid Medical Care Plan

RICHARD H. THURM, M.D.*

In the country's Colonial period a lazaretto was an institution built to quarantine victims of yellow fever, smallpox, and other epidemic diseases, in order to protect the citizens, rather than primarily for the care of the patient. The earliest hospitals were established for the military, or as medical departments of the almshouses where the poor were quartered. The provision for the needy was an early concern in the colonies. The first almshouse was built in 1662 in Boston, Massachusetts, 32 years after the city's founding (1).

The first organized hospital for the care of the sick, research into the causes of disease and its treatment, and the training of health professionals was developed at Philadelphia, Pennsylvania in 1751 as the Pennsylvania Hospital. Institutions were subsequently established at New Orleans, Louisiana (the Charity Hospital, in 1784) and in New York, N.Y. (the New York Hospital, in 1791) (2). The country's fourth oldest hospital, and the first in New England, was founded on Castle Island in Boston Harbor in 1799 as the Boston Marine Hospital. The latter has evolved over the years into the U.S. Public Health Service Hospital.

The concept of a formalized system of medical care for seamen can be traced to 1694 when, following the defeat of the French at La Hogue, an unfinished royal palace at Greenwich, England was converted to a home and hospital for "worn out and wounded seamen." Construction of additional naval hospitals at Chatham, Plymouth, and Portsmouth were started in 1745. These were partially supported by a deduction of sixpence a month from the seamen's wages to a trust fund established in 1581 known as the Chatham Chest. The name came from the fact that the money collected was kept in a large iron-bound chest at the Chatham Parish Church (3).

The American colonies' interest in the welfare of seamen resulted in the establishment, in 1708, of a hospital for seamen at the fort at Point Comfort near Norfolk, Virginia. In 1742, the Boston Marine Society was founded to "both promote interest in navigation and to assist and provide for needy and unfortunate mariners and their families." This group of shipmasters, merchants, and citizens, interested in the welfare of seamen, was organized as the Fellowship Club and incorporated in February 1754 as the Boston Marine Society. In 1768, the Marine Society of the City of New York was organized. Following the Revolutionary War, both the Commonwealth of Virginia and the State of North Carolina provided for a tax on the pay of all seamen in the state to be collected for medical care and hospitalization (4). The Annals of the first Congress note that during the opening session, on July 20, 1789, it was "ordered that a committee be appointed to bring in a bill or bills providing for the establishment of hospitals for sick and disabled seamen."

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In the immediate post-Revolutionary period Boston occupied a narrow peninsula connected to the mainland by a neck of land. It was the chief maritime city, benefiting as the nearest major port to Europe. The expanding foreign commerce was matched by the allied industries of ship building and fishing. Many of the young men of New England looked to the sea for their livelihood. The whole economy of the area was tied to the welfare of the seamen. It was therefore quite fitting that the Boston Marine Society should direct its attention to providing for the health of the men involved in this dangerous trade. A seaman disabled in the line of duty, or ill from exposure to disease in a foreign port or as a consequence of his inadequate diet, could expect very little in skilled attention. The Captain of the ship usually attempted to provide some measure of relief. If basic first aid proved of no value, the injured or sick seaman was left at the nearest port where it was hoped a family would care for him. Usually the town would confine him to the lazaretto, where very little care was provided.

An early effort to provide for such injured or ill seamen was an Act for the Government and Regulation of Seamen in the Merchant Service, approved by Congress on July 20, 1790, which required "that every ship or vessel belonging to a citizen or citizens of the United States, of the burthen of one hundred and fifty tons or upwards, navigated by ten or more persons in the whole, and bound on a voyage without the limits of the United States, shall be provided with a chest of medicines, put up by some apothecary of known reputation, and accompanied by directions for administering the same; and the said medicines shall be examined by the same or other apothecary once at least in every year, and supplied with fresh medicines in the place of such as shall have been used or spoiled; and in default of having such medicine chest so provided and kept fit for use, the master or commander of such ship or vessel shall provide and pay for all such advice, medicine, or attendance of physicians as any of the crew shall stand in need of in case of sickness, at every port or place where the ship or vessel may touch or trade at during the voyage, without any deduction from the wages of such seamen or mariner."

On October 12, 1790, a special meeting was called of the Boston Marine Society. Twenty-four members gathered at the Bunch of Grapes Tavern located at the corner of King (now State) Street and Mackerel Lane (now Kilby Street) (Fig. 1). This tavern had an excellent reputation as the "best punch house in Boston" (5). The first order of business dealt with a communication from General Benjamin Lincoln, the Collector of Customs at the Port of Boston, regarding "the building of a cutter for the protection of the trade." A draft of a letter or reply indicating the recommended size of the vessel was prepared. It is interesting to note that this marks the beginnings of the United States Coast Guard. The final item of discussion related to the appointment of a committee to consider the establishment of a marine hospital and to make

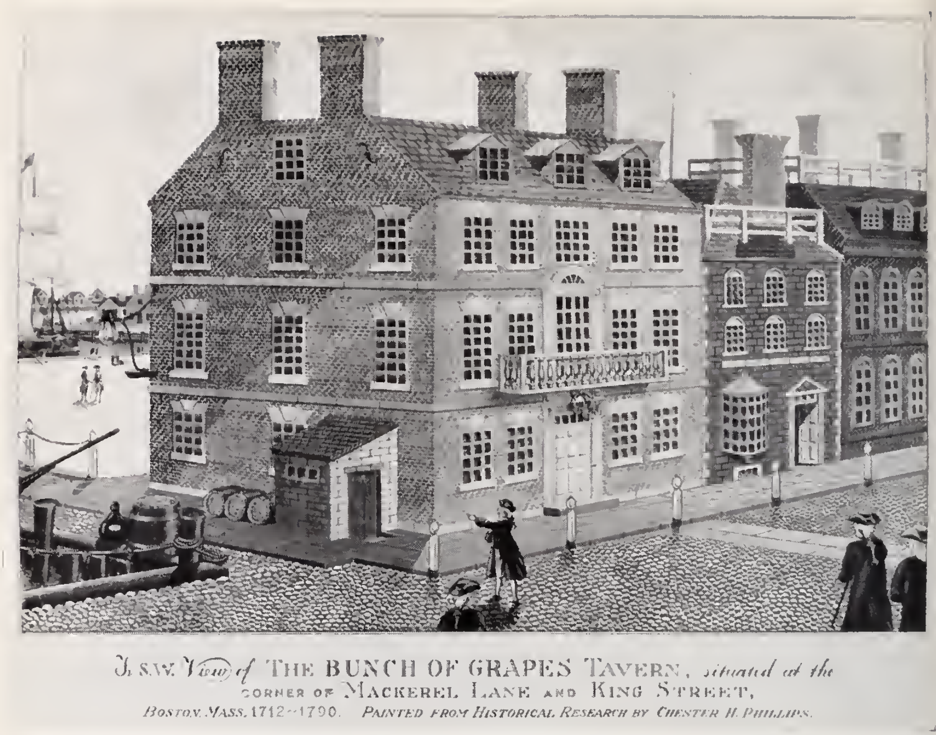
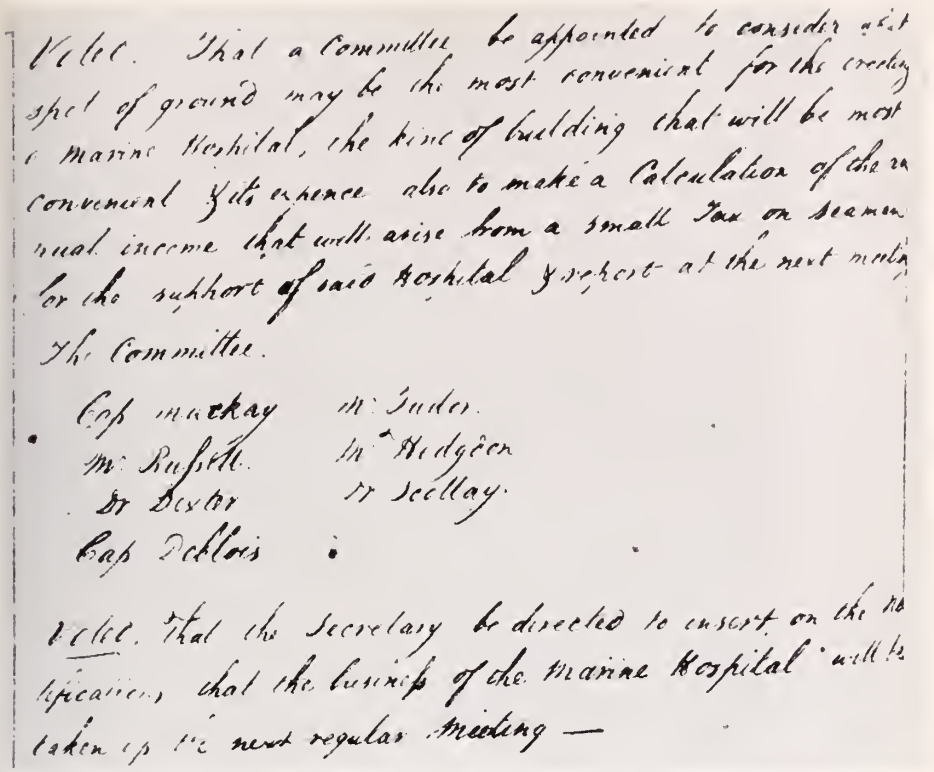

A S.W. View of THE BUNCH OF GRAPES TAVERN, situated at the
CORNER OF MACKEREL LANE AND KING STREET,
BOSTON, MASS. 1712—1790. PAINTED FROM HISTORICAL RESEARCH BY CHESTER H. PHILLIPS.

Fig. 1. The Bunch of Grapes Tavern.

a calculation of the tax to be levied on the mariners' pay to support the proposed institution (Fig. 2). A report was called for at the next regular meeting of the Society, which occurred 3 weeks later on Tuesday, November 2, 1790, with 22 members in attendance. The committee reported that some spot on the heights of Charlestown, East of the Town of Boston, was the most eligible situation for such a building. The recommendation was accepted and the committee was further instructed to draw up a petition to Congress for a hospital at that location, with the means of supporting it also being included. General Lincoln was then proposed for membership and was unanimously elected (Fig. 3). Two months later on January 4, 1791, the committee again reported on the subject of the hospital, drawing up a petition to Congress and sundry letters which were accepted and ordered to be sent forward (Fig. 4).

The petition and letters were duly forwarded and acted upon by the Second Congress, convening in October 1791 at Philadelphia, Pennsylvania. A committee was appointed which did present a bill "for the relief of the sick and infirm seamen." The Annals of Congress for Monday, January 21, 1793 record that in the House of Representatives, "Mr. Williamson, from the committee appointed, presented a bill for the relief of sick and infirm seamen; which was read twice, and committed." The measure was not considered further at that time despite the support of Alexander Hamilton, Secretary of the Treasury,



Voted. That a Committee be appointed to consider what
spot of ground may be the most convenient for the erecting
a Marine Hospital, the kind of building that will be most
convenient & its expence also to make a Calculation of the an
nual income that will arise from a small Tax on Seamen
for the support of said Hospital & report at the next meeting

The Committee.

Capt Mackay   Mr Tudor.
Mr Russell.   Mr Hodgdon
Dr Dexter   Dr Scollay.
Capt Deblois

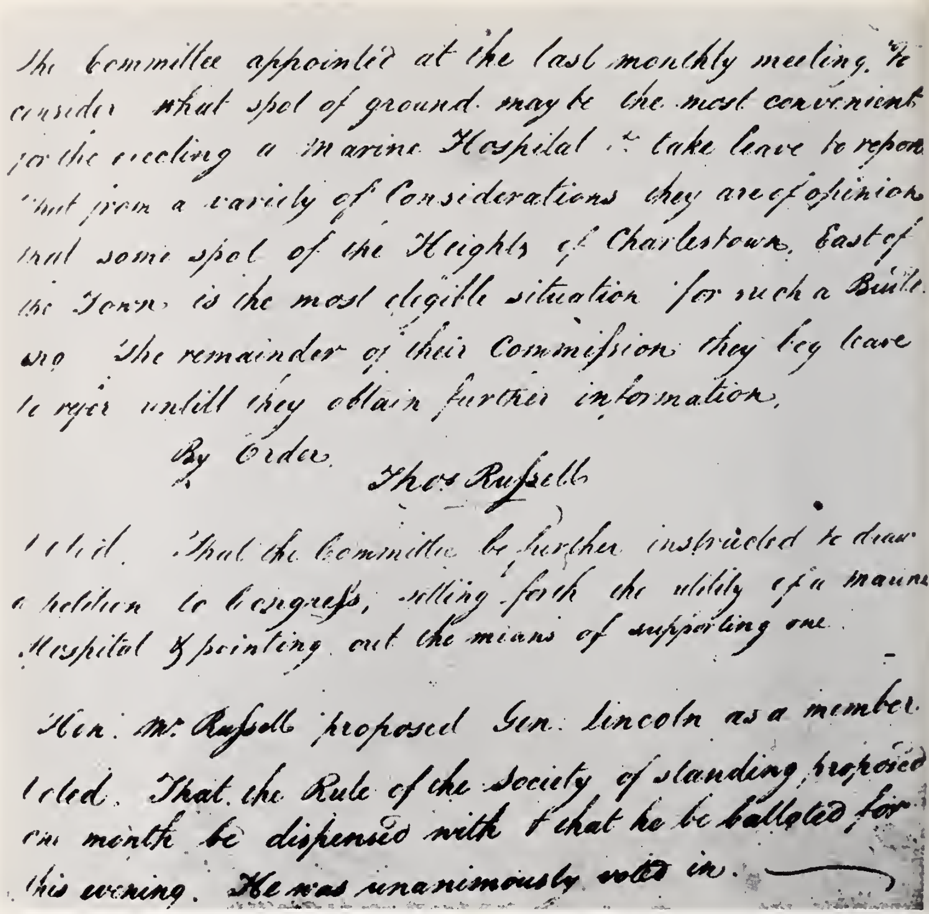
Voted. That the Secretary be directed to insert on the no
tifications that the business of the Marine Hospital will be
taken up the next regular Meeting —

FIG. 2. Minutes of meeting of the Boston Marine Society, October 12, 1790.

who recommended that "the establishment of one or more marine hospitals in the United States is a measure desirable on various accounts." A tax of ten cents a month from each seaman was suggested to support the program.

The issue was reopened in the House of Representatives of the Third Congress (1793–1795). On Friday, December 13, 1793, the Annals of the House of Representatives note that it was ordered "that a committee be appointed to prepare and bring in a bill for the relief of sick and disabled seamen; and that Mr. Goodhue, Mr. Watts, Mr. Nicholas, Mr. Winston, and Mr. Malbone, be the said commitee."

It wasn't until February 28, 1798 that the Annals of the Fifth Congress note that "Mr. Livingston, from the Committee of Commerce and Manufactures, reported a bill for the relief of sick and disabled seamen, and a bill declaring the consent of Congress to an act of the State of Massachusetts, in respect to certain piers; which were committed to Committees of the Whole." Action, so long delayed, ensued promptly. Eight days later the bill was brought to the floor of the House, where it was discussed. A proposal that a prepayment plan be incorporated into the measure necessitated that the bill be returned to the committee, the Annals reporting that "On motion of Mr. Livingston, the House resolved itself into a Committee of the Whole on the bill for the relief



The Committee appointed at the last monthly meeting to consider what spot of ground may be the most convenient for the erecting a Marine Hospital &c take leave to report that from a variety of Considerations they are of opinion that some spot of the Heights of Charlestown, East of the Town, is the most eligible situation for such a Building. The remainder of their Commiſsion they beg leave to refer untill they obtain further information.

By Order. Thos Ruſsell

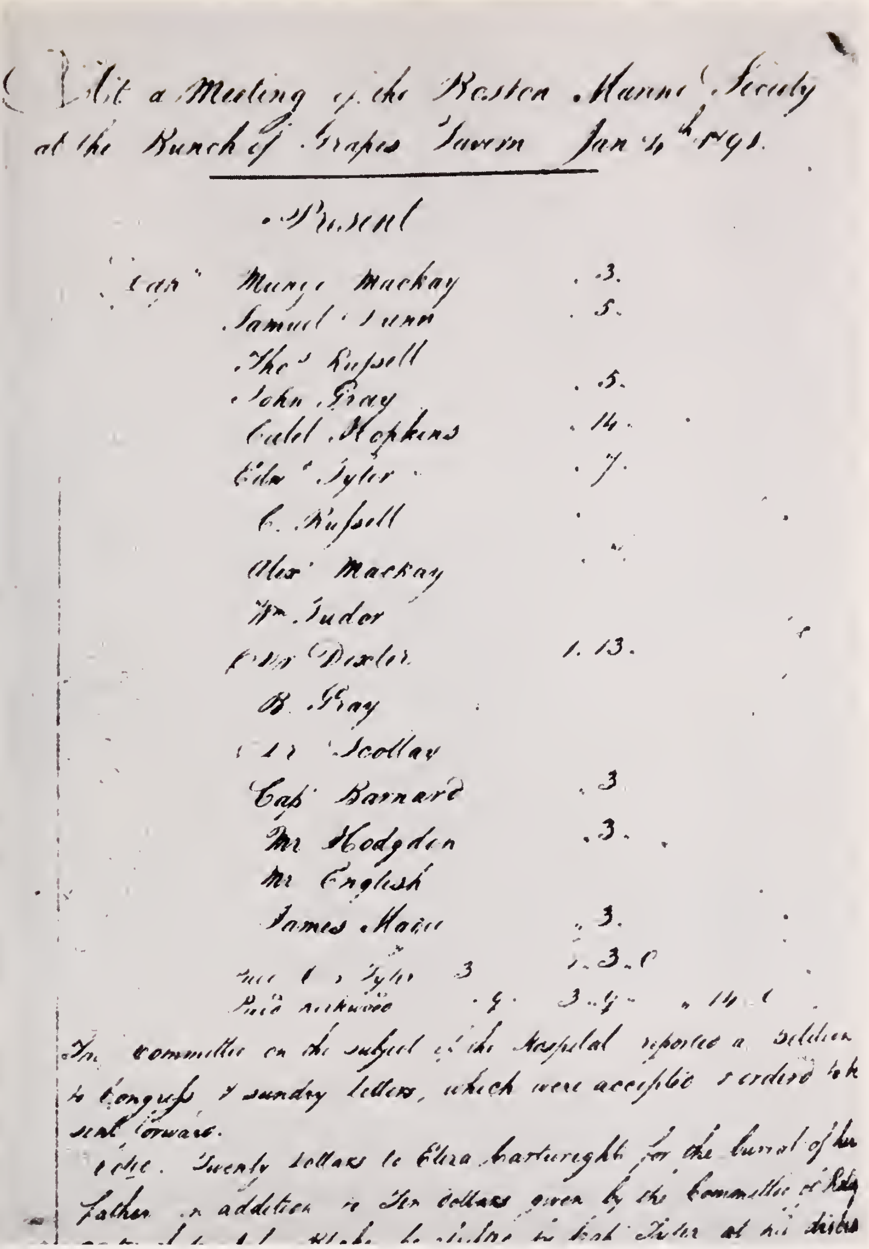
Voted. That the Committee be further instructed to draw a petition to Congreſs, setting forth the utility of a marine Hospital & pointing out the means of supporting one.

Hon. Mr Ruſsell proposed Gen. lincoln as a member.

Voted. That the Rule of the Society of standing proposed one month be dispensed with & that he be balloted for this evening. He was unanimously voted in.

Fig. 3. Minutes of meeting of the Boston Marine Society, November 2, 1790.

of sick and disabled seamen; when, after some conversation on the subject of the Marine Hospital, proposed to be established by this bill, as to the best manner of securing a support of it from the whole body of sailors of the United States, a proposition was adopted, enacting, 'that every owner or master of a vessel arriving from a foreign port into any port of the United States, shall, before the vessel is permitted to be entered, pay—cents per month during their voyage, for every man whom he has on board his vessel, which he shall be authorized to retain out of their wages.' The committee then rose, and the bill was re-committed to the select committee, to make it conformable to this principle." On April 9, 1798, on a motion by Mr. Edward Livingston, a member of the House of Representatives from New York, "the House went into a Committee of the Whole on the bill for the relief of sick and disabled seamen, and, after adopting some amendments, the bill was agreed to, and ordered to be read a third time tomorrow (this bill proposes the payment of twenty cents a month by every seaman in the United States towards the support of hospitals



At a Meeting of the Boston Marine Society
at the Bunch of Grapes Tavern Jan 4th 1791.

Present

Capt	Mungo Mackay	.3.
	Samuel Dunn	.5.
	Thos Russell	
	John Gray	.5.
	Caleb Hopkins	.14.
	Edwd Tyler	.7.
	C. Russell	
	Alexr Mackay	
	Wm Tudor	
	[illegible] Dexter	1.13.
	B. Gray	
	[illegible] Scollay	
	Capt Barnard	.3
	Mr Hodgdon	.3.
	Mr English	
	James Magee	.3.
		1.3.0
	[illegible] Tyler 3	
	Paid [illegible] .4.	3.4. .14.

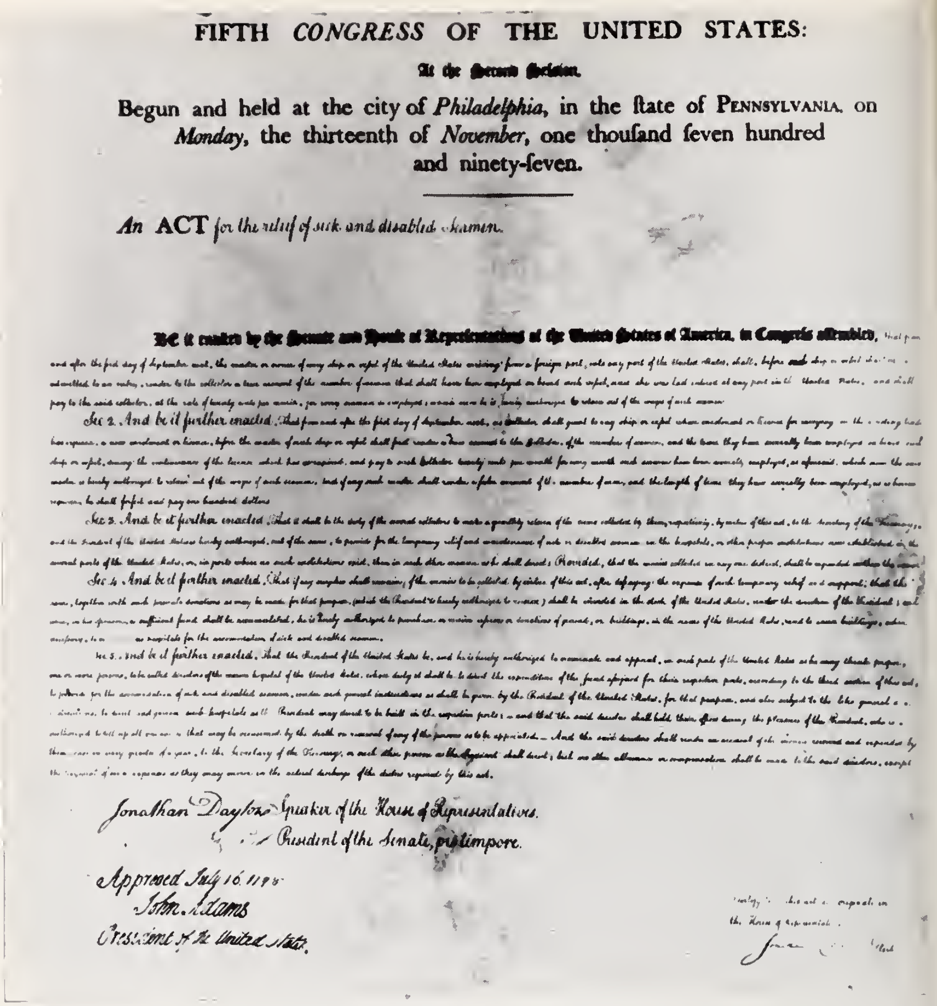
The committee on the subject of the Hospital reported a petition to Congress & sundry letters, which were accepted & ordered to be sent forward.

Voted. Twenty dollars to Eliza Cartwright for the burial of her Father in addition to Ten dollars given by the Committee of [illegible]

[illegible]

Fig. 4. Minutes of meeting of the Boston Marine Society, January 4, 1791.

for sick and disabled seamen)." The bill was debated on the following day with Mr. Samuel Sewall, a representative from Massachusetts, leading the opposition. "Mr. Sewall said, he was unwilling to say anything against this bill; but the passing of it would be attended with many inconveniences and objections, which ought to be mentioned. He allowed the objections principally applied to



FIFTH *CONGRESS* OF THE UNITED STATES:

At the Second Seſſion.

Begun and held at the city of *Philadelphia*, in the ſtate of PENNSYLVANIA, on *Monday*, the thirteenth of *November*, one thouſand ſeven hundred and ninety-ſeven.

An ACT *for the relief of sick and disabled Seamen.*

BE it enacted by the Senate and Houſe of Repreſentatives of the United States of America, in Congreſs aſſembled,

Jonathan Dayton Speaker of the House of Representatives.
President of the Senate, pro tempore.

Approved July 16, 1798.
John Adams
President of the United States.

Fig. 5. The Act of July 16, 1798.

the part of the Union from whence he came, where provision is already made for sick and disabled persons of every description, sailors as well as others, with which every person in the community is charged. Seamen in that quarter, therefore, now pay their full proportion of money for public charity; and if they were again to be called upon by this act, they would be made to pay in two different ways for the same object, without any advantage to themselves, because they are already entitled to relief in case of sickness or disability. But if this was not the case, he doubted the propriety of taxing seamen only for the support of what ought to be considered as a public charity. He thought the laws of reason and charity called upon the public at large in support of unfortunate men of this description, and that the burden ought not exclusively to be laid upon them. We have no common feeling with these men; the tax will fall upon no member of this House, but will be exclusively drawn from the earnings of a small part of the community, who, in all probability, will receive no advantages from it for fifty years to come, as large and splendid buildings must first be erected, in order to exhibit to the world a specimen of public charity. Nothing of this kind was to be seen in his part of this country; but this class of persons nevertheless were provided for. Neither native nor foreigner was there sick without care and relief being afforded him. It was difficult, therefore, to conceive upon what reasonable ground the seamen of his State could be called upon to contribute to a fund of this kind. The only reason which had been assigned was, that the New England seamen frequently visited the Southern States, where, if they were taken sick, there was no provision for their relief. He hoped those States would provide relief for such unfortunate persons; if not, sailors must go there as they go into foreign countries."

The provisions for sick and disabled persons of every description mentioned above by Mr. Sewall referred to a proposed hospital on the island of Martha's Vineyard, Massachusetts. This institution was used for a few years in the early 1800's (6).

The record for the House of Representatives for Thursday, April 12, 1798 reports that "The bill for the relief of sick and disabled seamen, after considerable opposition from Messrs. Sewall, Otis, and Reed, was passed, 59 votes being in its favor." The Proceedings for the Senate indicates that action on the bill ensued the following day: "The bill, sent from the House of Representatives, for concurrence, entitled 'An act for the relief of sick and disabled seamen,' was read the second time, and referred to Messrs. Goodhue, Langdon, and Read, to consider and report thereon to the Senate." Two months later, on Tuesday, June 19, 1798, it was recorded that "Mr. Goodhue, from the committee to whom was referred the bill, sent from the House of Representatives for concurrence, entitled 'An act for the relief of sick and disabled seamen,' reported the bill amended; and the amendments were read. Ordered, That they lie for consideration." It is of interest to note that Mr. Goodhue served as Chairman of the committee considering this bill in both the House of Representatives and Senate. Benjamin Goodhue, a merchant from Salem, Massachusetts, was elected as a Federalist to the first four Congresses as a Representative in the House. He was elevated to the Senate in 1797.

The bill was finally passed by the Senate on Saturday, July 14, 1798. The following was recorded in the Senate Proceedings of that date: "The Senate resumed the consideration of the report of the committee, to whom was referred the bill, sent from the House of Representatives, entitled 'An act for the relief of sick and disabled seamen,' and, the same being adopted, the bill was read the third time. Resolved, That this bill pass with amendments."

The bill was signed into law 2 days later by President John Adams, a member of the Boston Marine Society. The Act directed the master of every United States ship entering a United States port to render a true account of the number of seamen aboard since the vessel last entered a port of the United States, and to pay to the Collector of Customs a sum equal to 20 cents a month for each member of the crew, to be deducted from the wages of the seaman. Hospitals were to be bought or erected and directors appointed in the principal ports by the President to provide for the treatment of seamen. This was the country's first prepaid system of medical care (Fig. 5).

A subsequent act of March 2, 1799 included naval personnel, with provision for the deduction of 20 cents a month from the pay of officers and men beginning in September of 1799. The funds were turned over to the Secretary of the Treasury to support the Marine Hospitals. The men of the United States Navy were included until February 1811, when the Act authorizing the Navy to establish its own medical facility was passed by the Congress.

The original act stipulated that funds collected in each port had to be spent within that state. On March 2, 1799, the bill was amended to allow expenditures in neighboring states. A further alteration in 1802 created a common pool to finance the operation of all the marine hospitals. This unwieldy deficit financing continued for an additional 68 years when, in 1870, the monthly deduction from each seaman's wage was doubled to 40 cents. In 1884 the tax on seamen's wages was replaced by a tax on tonnage. Since 1905, Congressional appropriations have become the means of support for the hospitals.

Acknowledgments

The minutes of the Boston Marine Society (Figures 2, 3, and 4) were provided through the courtesy of Captain Chester L. Jordan, Treasurer of the Society.

The copy of the Bunch of Grapes Tavern painting is from the State Street Bank of Boston.

The historical background relating to Congress was quoted from the Statutes of the United States and the Debates and Proceedings in the Congress of the United States, compiled by Joseph Gales, and published by Gales and Seaton, Washington, D.C. in 1834. The Act of July 16, 1798 (Fig. 5) is from Chapter 77, Statutes 1, 1.605.

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Medicine's Responsibilities*

DICKINSON W. RICHARDS, M.D.†

Dr. James, Mr. Steinbach, Dr. Arnold Katz, Dr. Phyllis Katz, Dr. and Mrs. Louis Katz, Members of the Mount Sinai School of Medicine, Ladies, and Gentlemen:

I am greatly pleased, and greatly honored, to speak to you on this occasion of the Investiture of Dr. Arnold Katz as Philip and Harriet Goodhart Professor of Medicine.

First, may I congratulate Dr. Katz, and equally, the Mount Sinai School of Medicine, on this distinguished appointment? It is a measure of the vigor of an institution, and especially a new institution, that it finds generous support for new appointments and new ventures, a measure of its imagination and judgment that it has chosen so well, and a measure of its appreciation of this today, that it has made this Investiture so special and so gracious an occasion.

Dr. James has given you the major events in Dr. Katz's career. I should like to tell you something more about him.

Arnold Katz was born in Chicago into an intellectual and highly stimulating family environment. If I may say so, it is hard for me to imagine how one household could contain, and still hold its four walls together, two individuals as dynamic, exuberant, and irrepressible as Dr. Louis Katz and his son Arnold, but it obviously did, and with spectacular success. It is a special pleasure for me to greet Dr. and Mrs. Louis Katz, for whom I suspect this is an even more auspicious and delightful occasion than for anyone else. I have known Dr. Katz and held him in high regard and affection for very many years, which will be apparent, I think, as I proceed.

As you have heard, young Dr. Katz graduated from the University of Chicago in 1952, and from Harvard Medical School, cum laude, in 1956. I think it is true that most research workers who are really going anywhere get started early. This was certainly so with Dr. Katz. While still in college, he was a summer associate there in physiology, and during the summer of his graduation he worked with Dr. John F. Perkins in Woods Hole. A good abstract was published on the work of the first summer, and three of them, on the subject of smooth muscle, from the Woods Hole work.

In the summers of his 3rd and 4th years in medical school, he worked with his father in the Michael Reese Hospital, and three papers, on coronary blood flow and myocardial work, came out of this.

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* Remarks delivered at the Investiture of Dr. Arnold M. Katz as Philip and Harriet Goodhart Professor of Medicine, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029, September 26, 1969.

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Between his internship and his assistant residency at the Massachusetts General Hospital came 2 years with Dr. Christian Anfinsen at the National Heart Institute, and here Dr. Katz was introduced to the principles of protein chemistry which were to be the foundation of his later career.

In 1960 he found time for a clinical year with the late Dr. Paul Wood in London, that extraordinarily brilliant teacher of cardiology, and impetuous and delightful personality.

Then came the 3 years with Dr. Wilfried Mommaerts in Los Angeles, in which Dr. Katz began and went on to make the first of his signal contributions in the chemistry and physiology of heart muscle, ten papers and five abstracts being published on this research. A series of chapters and book reviews, written at the same time, on both clinical and physiological subjects, indicate his remarkable productivity and range of interests.

The work with Dr. Mommaerts was biological science of a fundamental nature, and it is not surprising that Arnold Katz's next move was to a department of physiology, this being at Columbia College of Physicians and Surgeons, with Dr. John V. Taggart. This was also extremely pleasant for us at Columbia, not only the professional association with Dr. Katz, but the opportunity to meet his wife and family as well. For myself, it was gratifying to find still another interest of them both, and this was ancient medicine, especially Greek. Mrs. Katz is in fact a classical scholar, having recently received her doctorate in Greek poetry.

During these and the next following years, Dr. Katz pursued his original and most skillful experiments with the contractile proteins of the heart, demonstrating, among many other things, the basic dependence of their activity upon the cations calcium, potassium, and sodium.

But the appeal of medicine has proved stronger than physiology. Dr. Katz moved from Columbia to the Department of Medicine at the University of Chicago, with Dr. Hans Hecht. His 3 years there have now been succeeded by the appointment of the Goodhart Professorship, which we celebrate here today.

In his wide range of interests, and in his predilection for medicine, Dr. Arnold Katz also follows Dr. Louis Katz, his father, who has been equally distinguished as a physiologist, medical scientist, teacher, and clinician.

It is the broad conception of medicine exemplified by these two men that has led me to a few thoughts on medicine's responsibilities. On this huge subject, obviously, I cannot try to present more than 2 or 3 aspects, and these in a very limited way.

The personal care of the sick remains the first of all responsibilities. Both Doctors Katz have stressed this many times, by word and deed, and so have I. If we need a final authority, we find Hippocrates, as he strove mightily to wrest medicine out of the hands of the philosophers, saying in the simplest words, "Medicine is concerned with sufferings of ordinary people, when they are sick or in pain"—and the word he uses is δῆμος, "demos," plain folk. So I need say no more about this.

Also, at the other end of the spectrum, I am not going to discuss the realm of medical science. This is being well taken care of, and its past achievements and future opportunities known to all. If more were needed, it has been supplied most clearly by the remarks of Dr. Katz, which you have just heard.

I have a few comments on Medicine and Society. We should remind ourselves that this subject, though newly emphasized in this country, is not new. The Scandinavian countries, with their magnificent institution of socialized medicine, have been providing the best of medical care to all their citizens, in a program that was undertaken more than half a century ago (1); this gives them, incidentally, the best health records in the world.

Achievements in this country, at city, state, and federal levels in the past decade have been considerable, as everyone knows, though vast problems and inequities remain. In the same period we have been reaching toward a reorientation in our medical school and hospital teaching, in the direction of community care. But we have had a long way to go. Our medical schools in the Flexner era, from, say 1920, until now, were set up as "citadels of excellence," for the advancement of medical science, and with little concern for the general health, and less for the immediate community. This may be an oversimplification, but it is undeniable that our teaching emphasis, for the last half century, has been on scientific medicine and the mechanisms of disease; there is no doubt, also, that the achievements have been magnificent and have fully justified themselves. But when our governments and communities, and now most earnestly our students, demand that we teach more about social problems and do more about community care, we can see that they are right.

The lack of emphasis on medical care, as such, exists within our institutions as well as outside. Just as one example, last year at the College of Physicians and Surgeons, one of our 2nd-year medical students, Henry Palmer, organized one of our regular clinics on the subject of "The Dying Patient." He brought in three excellent speakers, two from Chicago and one from Boston, and the session was both interesting and profoundly moving. My point is that this was for us not only an innovation, but something of a revelation as well, showing us clinicians how little we have been teaching our students about this most universal and most grievous of clinical events. I could continue on this subject of clinical teaching, but your imaginations can fill it in.

Now, finally, I should like to engage your attention, for my remaining minutes, on a somewhat larger scene that includes medicine, science, and society all together. This will require a certain amount of historical background, in medicine and scientific philosophy generally.

I am going back again to Hippocrates, and specifically, to his idea about Nature, the concept conveyed by the Greek word φύσις. This word comes from φύω, to grow, spring forth, come into being; it includes all nature and the natural order, and also the nature or constitution of man himself. Hippocrates (2) believed in the natural order of things, and believed that the function of medicine is to assist Nature to restore to man the balance of forces which is the state of health. This is not bad, I submit, for the 5th century B.C.; we could do today with more credit given to nature and less to man's manipulations. To put the case in most general terms, according to Hippocrates, man belongs to Nature, but Nature does not belong to man. I hope that Dr. Arnold and Dr. Phyllis Katz will not disagree with this interpretation, because it is central to my argument.

Hippocrates thus rejected the more anthropocentric philosophies of his day. Least of all, we can conclude, would he have accepted the Judaic, and later Judeo-Christian doctrine of man, under a beneficent Deity, as Lord of all Creation and all Nature.

There is an immense history of the varying fortunes of these two strongly contrasting attitudes toward Nature: man as part of Nature, and man as Nature's master. We cannot follow all this, but let us move down the ages to the 17th and 18th centuries of our era. Whitehead (3) has said that the Middle Ages were the Age of Faith, based on reason, and the 18th century the Age of Reason, based on faith. It was faith in science and its limitless possibilities, faith also in man and his limitless capability. There was a resurrection, or justification if you like, of the Judeo-Christian doctrine of man under a beneficent Deity, as Lord of Creation. Sometimes the Deity was left out, but the principle was the same. Man was the master, Nature in its limitless profusion was his servant. There was something also of the Crusader added to the conception, man conquering the infidels of a baleful Nature, or at least of the baleful and anti-human features of man's environment. In any case, the prospect was pleasing. The immense achievements of science and technology through the 18th, 19th, and early 20th centuries could not help but serve as proof of the validity of this conception.

Nowhere has this triumph been more evident than in medicine in the 20th century. Diseases had already become specific entities, with etiologic agents, cures, and preventions (4), and in the ever-strengthening anthropocentric philosophy of our age, they came to be looked upon as enemies to be put down and stamped out under the foot of the Lord of Creation. Each discovery was another battle won, in man's total conquest of Nature. One by one, infections, deficiency states, and metabolic defects, have been brought under control by miracle drugs or other agents; even chronic diseases alleviated. In our environment, pests unfavorable to our food products have been destroyed by powerful poisons; predatory animals have been wiped out. Heredity in plants and animals has been rearranged to man's benefit, and even in man, heredity is about to be placed under favorable control, at least in the minds of some.

With these, added to all the mighty advances in technology elsewhere in science, man has all but reached his goal as conqueror of Nature. Even two world wars, with their insane and ghastly devastation, did not disturb the dream, still resting in the confident scientific philosophy of the 18th century.

Then, slowly at first, afterward with increasing momentum, something happened. God disappeared from his heaven, and all was not right with the world. Man's power had become ever greater, but this, curiously, made matters worse and not better, because his power became too great for his own understanding and moved even further beyond his awareness of consequences. One physical event, man-induced, could reach around the world. There is now no place to hide, as many have said many times.

One is reminded of that moment in the life of Niels Bohr, that great and good man, when he awoke to the somber realization that the true and the good are not the same, that the true, in fact, can be appallingly evil. I refer, obviously, to the explosion of the first atomic bomb. The dreadful extensions of this event need no comment.

But beyond this, in his rash expenditure of power, man has pushed Nature beyond its capacity. Nature is no longer indefinitely extensive and extending, it is returning upon itself, and upon us. Man's unbridled use of his technological armament throws whole segments of the natural order out of balance, with the full meaning of this obscure, the outcome unknown.

In a word, the world has moved from an open to a closed system. This is something new for the world, or at least newly apprehended, and it must be reckoned with, because it is irrevocable.

Having announced this principle, one could easily cite as examples almost any of the world-shattering conflicts, disasters, and injustices that now torment us. This might or might not be justified, but I am going to be concerned only with a very few, and very familiar, man-made actions that are endangering the long-term natural order.

Among these, one is obliged to note first the increased radiation released by man-made technology. What will the strontium-90, with its half-life of 33 years, and now in our bones and reproductive tissues, have done to us in another generation, not to mention what further may be added? We are told that all is well, but who can be sure? There are, in addition, the effects of irradiation upon all the plants and animals which serve us so patiently.

There is also a curious affair, what might be called a Pentagonian euphemism, not so widely known, named "Broken Arrow." This is "accidental damage to a nuclear weapon resulting in actual or potential hazard to life or property." If Professor Larus (5) of New York University, writing in the *Saturday Review*, is correct, there have been a number of these, mostly from the crash of bombers and breakage of hydrogen bombs, releasing plutonium dust. This substance, enormously toxic, has a half-life of 24,000 years. The human system can tolerate no more than two billionths of a gram. There was such an accident in a rural area of northern Spain in 1966, and another in Greenland in 1968. There have been no reported accidental explosions of atomic weapons.

We used to have civilian conferences on nuclear explosions and what to do about them, but recently these have ceased, and we have essentially closed our minds.

To turn to another problem that we do talk about: DDT was hailed as a great discovery, and indeed it was. Who would have thought that it would persist and find its way into fish and bird life, to the extinction by sterilization of countless millions? It is also in ourselves. What will it do there? We don't exactly know. What other man-made chemicals which we have or will let loose on the world will be doing comparable things?

Take our widely polluted lakes and streams, with the deterioration of aquatic life down to the sea, caused by too much population and too many industries. This has been in progress for years, but now reaches intolerable proportions. How will it be controlled, and when? The same for the ravages of forests, waters, and minerals by many industries and many governments.

We applaud the peaceful uses of nuclear power. But what are the consequences of the heat and other effluent into nearby waters, and of possible diffusion of tritium into the air?

For medicine, as well as society generally, overpopulation is a dominant problem. The solution seems, at least, technically feasible, but socially still generations away. Yet the world cannot wait.

To sum up, going back to our basic social and scientific philosophy, if one is to be Lord of Creation, one must anticipate consequences and not wait for disaster to occur, then spend half of one's time and energy correcting past blunders. Man does not anticipate, and the less so, the more potent his weapon, and blunders continue.

If one is to be Lord of Creation, one must act for the good of all, not for a favored few. Man acts for a favored few. If one is to be Lord of Creation, one must not only foresee all, but count the cost. Man sees only a part, and does not, or cannot count the cost.

The plain fact is, man is not Lord of Creation, and never will be. Through his increasing and ill-used power, Nature can be greatly deranged, but not conquered, and if sufficiently deranged, may it not simply dispose of its one-time little conqueror?

> Man, proud man,
> Drest in a little brief authority,
> Most ignorant of what he's most assured,
> His glassy essence, like an angry ape,
> Plays such fantastic tricks before high heaven
> As make the angels weep.

This jeremiad in Shakespearean dress must seem to you to be a dismal way to end an address of welcome, congratulation, and good will; I should not have presented it to you if I did not believe, in the first place, that the proposition is important, that the dangers to our ecological survival, though more insidious, are at least as great as those to our social survival, that the problems are to be solved, not by the attitude of conquest and exploitation, but by the attitude of protection of nature and the natural order—man as the servant of Nature, in the Hippocratic tradition, and not its conqueror, and that medicine must take its part in this ideological, as well as practical, revolution. In the larger humanism, man must learn to conform, and not imperiously try to impose his will on things infinitely greater and more complex than he is. It is true that responsibility for all these things belongs to everyone, and medicine can take only a share.

There is an illuminating current report to Congress, with Harvey Brooks as Chairman, from the National Academy of Sciences, entitled "Technology: Processes of Assessment and Choice" (6). Professor Brooks puts the case in the broadest terms: "What is needed, clearly," he writes, "is a wide diffusion of deeper understanding about technology and deeper concern about its implications. Any change we recommend should, therefore, seek to induce as many different elements of society as possible to involve themselves in broadened assessment efforts."

I believe that medicine is well fitted for this responsibility. Certainly in the vast problem of over-population, where social survival and ecological survival are joined, medicine must play a key part. We should be more enterprising and more determined than we are. In the uphill struggle against industrial and military-industrial conquest of natural resources, we can also share, more than we do. It would be a good thing, for medicine and for society, if more physicians became more aggressive as citizens of the world in these endeavors.

What I am arguing for is not a program—I have no program—but a state of mind, one that demands an insistent concern over consequences. I believe that we shall have to partition our minds, retaining our enthusiasm for the advancement of knowledge, yet tempering this by a sort of stop-and-search: a constant inquiry into remote ill effects, and determination to correct these when they are found.

Harvey Brooks, again, puts the case elegantly: "Innovation," he writes, "whether technological or social, requires a certain measure of faith and missionary zeal, a willingness to proceed despite apparent problems whose solutions are not yet foreseen. There is a delicate balance between such faith, on the one hand, and rational analysis, on the other, a balance that must not be tipped too far either way."

This, I think, is the essence of what is before us today. This is how we should think and act. What is more, we should get on with it right away. The need is urgent.

As Hippocrates has reminded us, "The art is long, and life is short."

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Galactorrhea in Primary Hypothyroidism:

Report of Two Cases and Review of the Literature

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Spontaneous secretions of milk from the breasts of nonpregnant females may be due to a variety of causes. Thus, nonpuerperal galactorrhea may be associated with tumors of the adenohypophysis, hypothalamus, ovary, and adrenal cortex, exogenous administration of a variety of drugs and hormones, pituitary stalk secretion, and stimulation or trauma to chest wall or cavity.

Although relatively uncommon, disturbances in thyroid function may be associated with galactorrhea. Van Wyk and Grumbach (1) described a syndrome of juvenile hypothyroidism, precocious menstruation, and galactorrhea, and considered this an example of hormonal "overlap" in the pituitary feedback mechanism. Isolated instances of primary hypothyroidism associated with galactorrhea have been reported (2–8). The purpose of this communication is to report two additional cases of primary hypothyroidism and galactorrhea, and to stress the prompt response of the latter to thyroid hormone replacement.

Case Report

Case 1. J.F., a 51-year-old married Caucasian female, was first seen in the City Hospital Center at Elmhurst's Endocrine Clinic on September 3, 1965, with generalized weakness and facial swelling of several years' duration. During the previous 2 years she noted paresthesias of the fingers, arthralgias, leg edema, formication, dryness of the skin, diffuse hair loss, huskiness of voice, dyspnea on effort, diminished hearing, cold intolerance, constipation, postoccipital headaches, dizziness, intermittent daytime sleepiness, bouts of forgetfulness, and periorbital puffiness. She also noted spontaneous milky discharge from the breasts. She denied visual disturbances, syncope, polyuria, and acral enlargement. There was no past history of hypertension, head trauma, or drug ingestion. Her menses had always been regular until the age of 45, at which time menses ceased abruptly, with minimal postmenopausal symptoms. She had three children and denied postpartum hemorrhage or complications. The remainder of her history and family history, were entirely noncontributory.

Physical examination revealed an obviously grossly myxedematous female (Fig. 1) with a carotenoid skin color. Bilateral lid edema and blepharoptosis were present. Dryness of the skin was noted, as were macroglossia, brawny leg edema, and some reduction of axillary hair. Funduscopy and visual fields were normal. The thyroid was not palpable. The breasts were pendulous, and significant galactorrhea was easily expressed from both nipples (Fig. 2). The pulse rate was 58/minute, and the blood pressure was 102/70. The liver was palpable one fingerbreadth below the right costal margin. Pelvic examination revealed normal external genitalia with a slightly atrophic vagina. The cervix was normal, and the uterus atrophic. The adnexae were negative. The relaxation phase of the Achilles reflex was markedly delayed.

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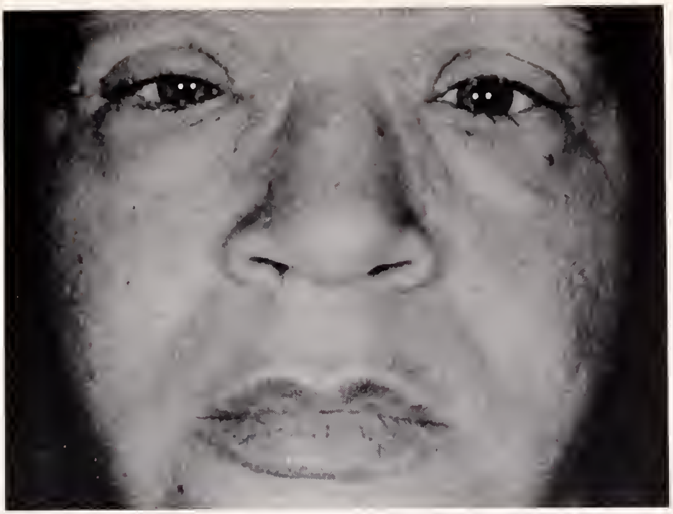

FIG. 1. Patient J.F., with gross myxedematous facies.

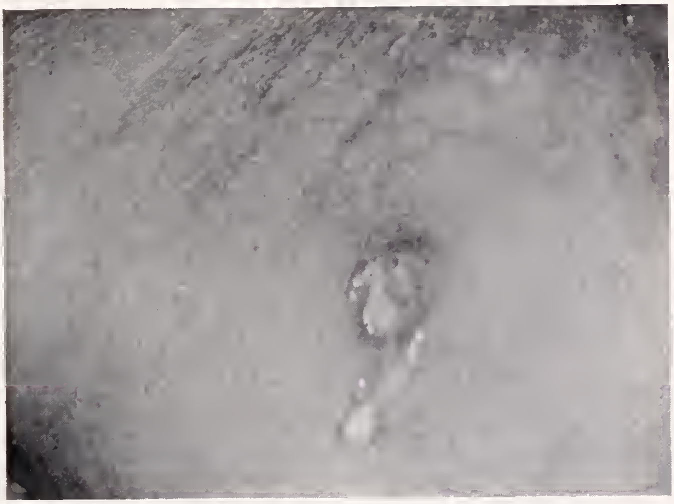

FIG. 2. Patient J. F.: Milky discharge from right breast.

Laboratory data included a white blood cell count of 9,100/mm^3, with a normal differential. The hemoglobin was 10.8 gm%, with an hematocrit of 33%. The erythrocyte sedimentation rate was 46 mm/hour (Westergren). Normal laboratory values were obtained for the fasting blood sugar, blood urea nitrogen, calcium, phosphorus, alkaline phosphatase, uric acid, cephalin flocculation, serum electrolytes, albumin, globulin, serum electrophoresis, and prothrombin time. The blood cholesterol was 270 mg%. Routine urinalysis was within normal limits.

The protein-bound iodine was 1.7 μg%. A 24-hour radioactive I-131 thyroidal uptake was 3%, with a rise to 7% following 3 consecutive daily injections of 10 units thyrotropin (TSH).

A 24-hour urine for gonadotropin was positive at 10 mouse uterine units, and the total neutral 17-ketosteroids and 17-hydroxycorticoids were 4.0 and 3.3 mg/24 hours, respectively.

Vaginal smear showed slight estrogen deficiency, and a Papanicolaou smear was negative for tumor cells.

Microscopic analysis of the milky secretion from the breasts with Sudan-IV stain showed numerous orange-staining fat vacuoles of varying size.

Roentgenograms of the sella turcica were normal. A chest x-ray demonstrated cardiomegaly, with no evidence of congestive heart failure. An electrocardiogram showed sinus bradycardia of 50/minute with low voltage of the QRS complex in all leads.

The patient was placed on gradual increments of thyroid replacement therapy, and no further breast secretions were noted 6–8 weeks following the initiation of thyroid therapy. A recent follow-up 2 years after treatment was instituted with 3 grains of thyroid extract daily and revealed her to be euthyroid with no complaints.

Case 2. L.C., a 44-year-old married Caucasian female, was admitted to the Bronx Hospital on January 14, 1965, complaining of fatigue and dryness of the skin. Her menses had been regular until 1 year prior to hospitalization, when increasingly heavy menstrual flow was noted. She had had five children with no postpartal complications. However, persistent lactation was noted since delivery of her last child in 1959. Additional complaints included paresthesias, increased weight, thinning of hair, and cold intolerance. Pertinent physical findings were moderate dryness of the skin and hair, congenital strabismus, pulse rate of 58/minute, and bilateral expression of milky fluid from both breasts. The thyroid was not palpable.

Laboratory data included an hematocrit of 34%, with a normal white blood cell count and differential. The erythrocyte sedimentation rate was 78 mm/hour. Normal laboratory values were obtained for the fasting blood sugar and an oral glucose tolerance test, blood urea nitrogen, serum electrolytes, calcium, phosphorus, alkaline phosphatase, serum albumin and globulin, and serum electrophoresis. The serum cholesterol was 285 mg%, while the uric acid was 6.6 mg%.

The protein-bound iodine was 2.7 μg%. A 24-hour urinary excretion of total neutral 17-ketosteroids and 17-hydroxycorticoids was 5.0 and 3.5 mg, respectively, while the 24-hour urinary gonadotropin titer was positive at 5 and negative at 10 and 100 mouse uterine units.

A plasma growth hormone assay by the radioimmunoassay procedure of Roth et al (9) was less than 0.5μ μg/ml.

Previous studies in March 1964 revealed a thyroidal I-131 uptake of 2%, with a 3% uptake following 3 consecutive days of intramuscular injection with 10 units TSH.

An electrocardiogram demonstrated sinus bradycardia with low voltage. Chest and skull roentgenograms showed no abnormalities.

Replacement with 3 grains of thyroid extract daily caused complete cessation of galactorrhea 3 months after treatment was instituted. A 3-year follow-up revealed the patient to be asymptomatic.

Discussion

Standard textbooks on the thyroid gland (10, 11) and a clinical review of 400 cases of hypothyroidism (12) make no comment on the occurrence of galactorrhea in hypothyroidism. Although this phenomenon is not frequent, galactorrhea in primary hypothyroidism does occur. The expression of milk from the breasts may lead the clinician to suspect intrasellar or parasellar tumors and other endocrine neoplasms (5, 6, 13–15). Recent clinical studies of plasma prolactin (6) have revealed elevated values in instances of Chiari-Frommel syndrome (16, 17) and pituitary tumors associated with galactorrhea. It has been postulated that the increased plasma levels of prolactin may be due to increased secretion of this hormone by the pituitary gland, or possibly by suppression of a hypothalamic prolactin inhibitory factor (PIF) (18, 19).

Although instances of abnormal lactation may occur as a result of the independent production of prolactin by intrasellar tumors or because of disruption of tonic hypothalamic influences on the anterior pituitary by mechanical, metabolic, or inflammatory processes, others may result from a pathophysiologic hormonal overlap in pituitary feedback. The latter results from an endocrine target-organ deficiency. In 1960, Van Wyk and Grumbach (1) described a syndrome of precocious menstruation and galactorrhea secondary to juvenile hypothyroidism. Three girls of ages 7, 8, and 12 years had enlargement of the sella turcica, and one had hyperpigmentation as well. The abnormal signs disappeared as they became euthyroid with replacement therapy, and the pituitary fossa decreased in size. These authors attributed this syndrome to a lack of specificity of the pituitary feedback mechanism, resulting in the secretion of gonadotropins, lactogenic, and melanocyte-stimulating hormones. Other cases have also been described in primary hypothyroidism with precocious pubertal development in females (7, 20–24) and precocious testicular maturation in males (25, 26). Although hormonal overlap in pituitary feedback is an attractive hypothesis, one may not entirely exclude altered end-organ sensitivity to pituitary tropic hormones.

A review of the literature, as well as the two cases described in this paper, lends support to the occurrence of galactorrhea in primary hypothyroidism. Careful clinical evaluation of pituitary function with standard pituitary function studies and roentgenograms is necessary to exclude secondary hypothyroidism. Documented instances of galactorrhea in primary hypothyroidism generally occur as persistently prolonged postpartum lactation (2–4, 6, 8). Characteristically, complete remission of galactorrhea follows thyroid replacement therapy (3, 4, 6–8). Patient J. F. of the first case of this report is of interest in that she is the first documented postmenopausal female with primary hypothyroidism and galactorrhea with prompt response of the latter to thyroid therapy.

Ross and Nusynowitz (8) described a 29-year-old female with primary hypothyroidism, postpartum galactorrhea, and amenorrhea. This case and three similar patients (3, 6, 15) revealed no evidence of pituitary tumor. In three of these reports (3, 6, 8) thyroid hormone replacement was followed by cessation of galactorrhea and resumption of normal menses. The presence of amenorrhea in these four cases may represent a distinct clinical entity and argue against hormonal overlap as a major etiologic factor (8). Indeed, Ross and Nusynowitz believe the abnormality to be due to inhibition by the hypothyroid state of the hypothalamic center controlling release of prolactin and luteinizing hormone (LH). Thus, primary hypothyroidism, especially occurring postpartally as well as postmenopausally, may be a condition under which LH production is inhibited and prolactin production is augmented.

It should be stressed that milky breast secretion in primary hypothyroidism is reversible with thyroid replacement therapy. This is an important clinical point in that galactorrhea in patients with secondary hypothyroidism is not likely to respond to this therapy. Since amenorrhea may accompany the hypothyroid state, consideration of the Forbes-Albright syndrome (14) or other causes of amenorrhea and galactorrhea must be rigidly excluded (27, 28).

The development of pituitary neoplasms in the presence of target organ hypofunction is not well documented. Although instances of adenohypophyseal tumors have been described in primary hypothyroidism (29–33), they have usually been associated with primary hypothyroidism of long-standing duration. Whether these tumors bear any relation to the hypothyroid state, or may have represented autonomous behavior in the setting of hormonal overlap, cannot be answered at this time.

Summary

Although relatively uncommon, disturbances in thyroid function may be associated with galactorrhea. Two females, aged 44 and 51 years, with primary hypothyroidism and galactorrhea are presented. There was cessation of galactorrhea in both subjects following thyroid hormone replacement therapy.

A review of several other instances of galactorrhea and primary hypothyroidism affords speculation regarding possible hormonal overlap in the pituitary feedback mechanism. The pathophysiology of abnormal breast secretion in relation to thyroid hypofunction is also reviewed.

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A Study of Major Postoperative Wound Sepsis

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Introduction

Twelve hundred ninety-five operations performed on The Mount Sinai Hospital Elmhurst affiliate General Surgical Service were reviewed during a 12-month period. The material following is presented because despite intensive efforts on the part of medicine to reduce the incidence of postoperative wound sepsis, a plateau appears to have been reached. The currently cited incidence in the surgical literature is a range of 1 to 22 percent (1–3).

The study seeks: (1) the documentation of the actual incidence of major postoperative wound sepsis; (2) a greater understanding of the genesis of postoperative wound sepsis; (3) an assessment of the importance of the endogenous environment (the patient himself) compared to the exogenous environment as a contributing factor to postoperative wound sepsis; (4) the elucidation of factors involved in enhancing or resisting infection once contamination has occurred; and (5) the application of more effective methods for treating infection once present.

Method

Whenever a postoperative wound infection was discovered, a culture was taken of the initial wound exudate for growth and sensitivity studies. A surgical resident was assigned the task of completing a data sheet (Fig. 1). Each patient was then checked by the author to confirm the presence of wound sepsis, and personally followed until resolution. In addition, the entire series of cases (infected and noninfected) was reviewed for the data established in Fig. 1.

Throughout the course of the study, at weekly intervals, blood agar plates were placed in two operating room suites and bacteriologic sampling was done before, during, and after surgery. The plates were placed at table level and at the entry points of the operating room suites, all plates being exposed for 1 hour and then incubated at 37°C for 24–48 hours in order to define a possible relationship between these organisms and the wound pathogens.

Definition of Terms

MAJOR POSTOPERATIVE WOUND INFECTION

To be considered in this category, the wound had to show a grossly purulent discharge; local wound management and/or systemic chemotherapy had to be instituted, and the wound infection had to contribute to prolonged morbidity.

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Name
Chart number
Age
Sex
Nutritional and metabolic factors
Diagnosis
Operation
Duration of operation
Wound closure
Drains
Infection site
Remote infection
Microorganisms and sensitivity
Antibiotics
Morbidity
Result

FIG. 1

Wound erythema, induration, and stitch abscesses were not included in this study.

WOUND CLASSIFICATION (4)

(1) *Clean wounds:* wounds in which the procedure was performed under aseptic conditions, without a major break in technique, in which the gastrointestinal tract, genitourinary tract, and tracheal-bronchial tree were not transected or resected, and which was not performed in the vicinity of any apparent inflammatory reaction; examples include herniorrhaphy, thyroidectomy, excision of soft tissue masses, and sympathectomy.

(2) *Clean-contaminated wounds:* wounds in which all of the above characteristics were true, except that opening or transection or resection of viscera known to contain bacteria has occurred; examples include small and large bowel resections, gastrectomy, and cholecystectomy.

(3) *Frankly contaminated wounds:* wounds in which obvious pus, abscess formation, and/or (with) continued gross spillage from a perforated viscus was encountered; examples include diverticulitis with perforation, ruptured appendix, perforated peptic ulcer, and subphrenic abscess.

Presentation of the Data and Discussion

In our series of 1295 operations there were 97 major wound infections, with a wound infection rate of 7.5 percent. Table I shows the infection rate on the basis of wound classification as defined. Frankly contaminated and clean-contaminated wounds, respectively, showed a sixfold and fourfold increase of infection compared with clean wounds, confirming the fundamental importance of increasing intraoperative wound contamination enhancing the risk of clini-

TABLE I

Postoperative Surgical Wound Infection Data from 12-Month Survey at Elmhurst

Wound classification	No. of wounds	No. of wounds infected	Infection rate (%)
Clean	758	22	2.9
Clean-contaminated	367	46	12.3
Frankly contaminated	170	29	17.0
Total	1295	97	7.5

cal infection. What is less obvious is the source of this contamination, and the determinants of wound infection or resistance to it once the contamination has occurred.

Source of Contamination: Endogenous Verses Exogenous

If wound contamination were primarily exogenous (either the operating room environment or the shedding of bacteria from personnel), there should be little or no difference in infection rate among the three wound classes. On the other hand, if endogenous contamination (the patient's skin, respiratory, alimentary, and genitourinary tracts) is of prime importance, then the marked difference in clinical sepsis among the three wound classes becomes more understandable.

Three facets supporting the endogenous source as primary in the genesis of postoperative wound sepsis were borne out in this study, including the following: (1) sources of infection; (2) remote infection; and (3) the microbiology of the infected wounds and the exogenous operating room environment.

As can be seen from Table II, 65 of the 97 infections resulted after surgery on the gastrointestinal tract. Forty-eight of the infections occurred after surgery of the large intestine and appendix. In all of these cases, the organisms cultured from the wounds were either gram-negative or enterococci, normal inhabitants of the healthy colon, including the coli-aerogenes group, Proteus, and Pseudomonas organisms. In 18 of 20 vascular procedures where distal infection was present (wet gangrene of the digits), polymicrobic infection or monomicrobic infection with gram-negative organisms was present. In two other polymicrobic infections, Staph aureus coagulase positive and a beta hemolytic streptococcus were cultured. Moreover, from the nine biliary tract cases and from the five stomach procedures, gram-negative organisms, indigenous to the gastrointestinal tract, or urinary tract pathogens were cultured.

TABLE II

Sources of Infection: 97 Operative Procedures

Intra-abdominal	69
Biliary tract	9
Colon	33
Appendix	15
Stomach	5
Small bowel	3
Exploratory laparotomy	4
Hernia	3
Vascular	20
Bypass grafts	7
Amputation	13
Radical mastectomy	3
Radical neck dissection	2
Total	97

TABLE III

*Incidence of Postoperative Wound Sepsis in Relation to Remote Infection**

Remote infection	No. of wounds	No. of wounds infected	Infection rate (%)
Absent	1145	60	5.2
Present	150	37	24.6
Total	1295	97	7.5

* The documentation of acute or chronic infection remote from the operative site and present prior to the operation.

In only two of the laparotomy cases, one radical mastectomy, and two of the radical neck dissections were gram-positive organisms cultured (Staph aureus coagulase positive, beta hemolytic streptococcus, and diphtheroids). Therefore, in 91 of 97 cases, the bacteria cultured were not from the exogenous environment, nor were they likely to be shed by operating room personnel (16); they were indigenous to the patient, emphasizing the importance of endogenous contamination as the prime reservoir of the subsequent infection.

Remote infection has been defined for the study as follows: the bacteriologic documentation of acute or chronic infection remote from the operative site, present prior to surgery, and the recovery of the same organism or organisms from the infected wound exudate. The presence of such remote infection was characterized by an almost fivefold increase in wound infection rate (Table III). Several clinical studies have shown that patients with a primary infection may develop postoperative wound sepsis with the same offending organism (5, 6).

In this series, 29 patients demonstrated microorganisms present in the urinary tract preoperatively or prewound infection which appeared subsequently in the infected wound (Table IV). In eight cases with peripheral vascular disease and wet gangrene of the toe or toes the organism present at this site subsequently appeared in the infected wound of the amputation site or in the incisions of a femoral popliteal bypass procedure. Two examples will demonstrate the phenomenon of autogenous seeding:

CASE REPORTS

Case 1. A.R., a 67-year-old white female, entered the hospital with intractable pain from a duodenal ulcer. The patient had a known chronic urinary tract infection with E. coli. This had been present for 1 year previously. The urinary tract was recultured prior to surgery and grew out E. coli again. The patient underwent a subtotal gastrectomy and a wound infection developed yielding E. coli as the pathogen. After intensive local therapy the wound healed. Three months later the patient was readmitted for open reduction and fixation of a fractured hip. Postoperatively the wound became infected and E. coli again was cultured as the pathogen.

Case 2. M.F., a 61-year-old white male with esophageal varices, entered the hospital for elective surgery. A severe bilateral purulent conjunctivitis was present, which grew out Pseudomonas aeruginosa as the pathogen. The patient ran a course of high fever, and blood cultures also grew out Pseudomonas aeruginosa. The infection was treated and

TABLE IV

Autogenous Seeding of Infected Wound from Remote Preoperative Primary Site

Source	No. of cases	Organisms cultured from urine preoperatively		Organisms cultured postoperatively from infected wound site		
		No. of cases		No. of cases: hernia	No. of cases: biliary tract	No. of cases: Gastrointestinal tract*
Urinary tract infection	29	14	Escherichia coli	1	4	9
		5	Aerobacter aerogenes		2	3
		2	Proteus mirabilis			2
		1	Pseudomonas aeruginosa			1
		7	Mixed gram-negative organisms		1	6
Source	No. of cases	Organisms cultured from gangrenous toe(s) preoperatively		Organisms cultured postoperatively from infected wound site		
		No. of cases		No. of cases: BK AMP	No. of cases: AK AMP	No. of cases: femoral popliteal bypass
Gangrene of toe(s)	8	3	Escherichia coli	1	1	1
		2	Aerobacter aerogenes, Proteus mirabilis, Escherichia coli		1	1
		1	Proteus mirabilis, staphylococcus Aureus coagulase positive	1		
		1	Proteus vulgaris, Pseudomonas aeruginosa, beta hemolytic Streptococci, Staphylococcus aureus coagulase positive	1		
		1	Pseudomonas aeruginosa		1	

* Five of these cases involved wound infection after subtotal gastrectomy (three cases of Escherichia coli, one case of Aerobacter aerogenes, and one case of Pseudomonas aeruginosa).

cleared prior to surgery. The subsequent operative wound following a portacaval shunt became infected with Pseudomonas aeruginosa. Similar phenomena were identified in the remaining 35 cases presented in Table IV. The isolation of almost all of the organisms in these 37 cases as gram-negative bacteria, and their remote origin, further emphasizes the importance of autogenous seeding in the genesis of wound sepsis.

The data presented in Figure 2 reveal that 176 organisms, or 93 percent of those cultured, were of endogenous origin. Staph aureus coagulase positive was cultured only eight times from the infected wounds, and in most of these cases it represented a suprainfection with the organism rather than the primary pathogen. This was determined in 5 of the 8 cases in which Staph aureus coagulase positive only appeared after 5 to 7 days of antibiotic therapy directed against a gram-negative pathogen that was present in the original wound exudate. Furthermore, in comparing the types of organisms in Figure 2 (from

A

Wound Organisms

Total number	188
Polymicrobic infection	71
Monomicrobic infection	26

B

Specific Organisms Recovered from Infected Wounds

Organism	No. of Cases
Staphylococcus aureus coagulase positive	8
Escherichia coli	58
Aerobacter aerogenes	34
Proteus mirabilis	30
Proteus vulgaris	6
Pseudomonas aeruginosa	18
Enterococci	21
Beta hemolytic streptococci	4
Other streptococci	8
Clostridium welchii	2
Bacteroides	1

FIG. 2

Weekly Recovery of Microorganisms from Operating Room Suites (The Exogenous Environment)

No. of Weeks Sampled	Organisms	No. of Weeks Recovered
52	Staphylococcus aureus coagulase positive	6
	Staphylococcus epidermidis	52
	Bacillus subtilis	46
	Diphtheroids	48
	Neiserria species	42
	Flavobacterium	12
	Gamma streptococci	16
	Streptococcus viridans	18
	Sarcina species	14
	Fungal organisms	26

FIG. 3

the infected wounds) with those in Figure 3 (from the operating room environment), it is clear that exogenous contamination played an insignificant role in wound infection in this study.

The Determinants of Wound Infection or Resistance to It Once the Contamination has Occurred

In Table I it can be seen that clean cases did become infected (2.9 percent), and that the majority of frankly contaminated cases (83 percent) did not become infected. This implies that clinical wound sepsis depends on the balance between wound contamination and patient resistance. Some general factors considered relevant to host defense were studied, including age, sex, and nutritional and metabolic factors (4):

AGE

Increasing susceptibility to infection in older individuals has been reported clinically by many investigators (3, 7, 8). This is probably related to de-

TABLE V

Incidence of Postoperative Wound Infection by Decade

Decade	No. of wounds	No. of wounds infected	Infection rate (%)
10–19	124	3	2.4
20–29	136	5	3.6
30–39	188	8	4.3
40–49	212	10	4.7
50–59	212	17	8.0
60–69	228	26	11.0
70–79	150	20	13.0
80–89	45	8	18.0
Total	1295	97	7.5

TABLE VI

Incidence of Infection by Sex

Sex	No. of wounds	No. of wounds infected	Infection rate (%)
Male	698	48	6.8
Female	597	49	8.2
Total	1295	97	7.5

creased host resistance to infection, with poor wound healing as a reflection of aging on the entire reparative process of injured tissues (9). Table V represents the incidence of wound sepsis by decades. The rate of infection is about eight times higher in the eighth decade as compared to the second decade.

SEX

A slightly higher percentage of infection was present in female patients, although this was not great enough to incriminate sex as a causative factor in postoperative wound sepsis (Table VI).

NUTRITIONAL AND METABOLIC FACTORS

Four specific conditions were studied: (1) diabetes mellitus; (2) steroid therapy; (3) marked obesity; and (4) severe malnutrition. The incidence of wound infection in patients manifesting any of these conditions is shown in Table VII.

Diabetes mellitus was associated with a fourfold increase in wound infection compared with the group as a whole. It is a common clinical impression that diabetics are more susceptible to postoperative wound infection (1). Scientific data to substantiate this are not complete. The following information is known: 1) The early granulocytic phase of the local cellular response is significantly delayed and diminished in patients with diabetic acidosis (10); 2) Animal experiments have demonstrated impaired wound healing in alloxan-induced diabetes (11); and 3) In normal tissues, the glycolytic activity of inflamma-

TABLE VII

Incidence of Infection in Relation to Certain Nutritional and Metabolic Factors

Factor	No. of wounds	No. of wounds infected	Infection rate (%)
None	1050	54	5.1
Diabetes mellitus	94	18	19.0
Steroids	18	3	17.0
Severe obesity	61	8	13.0
Severe malnutrition	72	14	19.0
Total	1295	97	7.5

tory cells results in the accumulation of bacteriocidal lactic acid, whereas in the diabetic, inflammation yields mainly keto acids which may favor bacterial growth, and can protect microorganisms against the toxic action of lactic acid (12).

Steroid administration was associated with a twofold increase in wound infection compared with the group as a whole. Several mechanisms for this depressive action of steroids on host defense have been postulated, including: 1) depression of antibody formation; 2) interference with local inflammatory reaction; 3) delay in movement of colloidal and particulate matter through the ground substance; and 4) depression of the reticuloendothelial system (13). Moreover, cortisone, by reducing the inflammatory reaction, is likely to result in the decreased production of lactic acid at the infection site (12).

Obesity was associated with a twofold increase in wound infection compared with the group as a whole. Adipose tissue is relatively avascular, and therefore has a lesser capacity for contamination resistance than other tissues. Recent studies have demonstrated that both blood volume and blood flow per unit weight are lower in adipose tissue than in lean tissue (14).

Malnutrition was associated with a greater than twofold increase in wound infection compared with the group as a whole. It has been demonstrated that acute starvation results in the utilization and depletion of glycogen reserves of the body; as phagocytic cells depend on glycogen for their metabolic activity, acute starvation may enhance infection potential through a depressive influence on phagocytosis, yielding a decreased amount of bacteriocidal lactic acid in the inflammatory areas (12). On the other hand, it has been shown that in chronic starvation, the preterminal nutritionally depleted patient with hypoproteinemia can synthesize large amounts of antibody and even have an abundance of circulating phagocytes (15).

The postoperative infection rate of patients with these conditions was significantly higher than for the group in which they were absent.

Other factors were also examined for their possible effect on infection rate:

WOUND CLOSURE

In this series of 97 wound infections, all wounds were primarily closed. The major suture materials used were, wire in 63 cases, chromic catgut in 21 cases, and silk in 13 cases. As wire was the material most frequently used in the closure of all types of wounds, it cannot be considered that this material is more conducive to wound sepsis. It is our impression that the type of suture material used is irrelevant to the development of postoperative wound sepsis; host factors, nutritional and metabolic factors, and the presence of remote infection are especially significant, as well as strict adherence to meticulous surgical technique.

DRAINS

Initially an attempt was made to analyze the incidence of infection in drained and undrained wounds. However, it soon became apparent that too many factors were involved to accurately interpret these data. For example, most wounds drained were clean-contaminated or frankly contaminated, an inherent source of contamination and potential infection possibly much greater than the introduction of microorganisms from the drain tract itself. The subtlety involved in the clinical evaluation by the operating surgeon to drain to protect a suture line or for questionable hemostasis, or for exiting possible further intestinal content leakage (an appendiceal stump, or bile leakage from the liver bed after cholecystectomy) could not be measured. One can only state that drained wounds were associated with a higher infection rate than undrained wounds (Table VIII).

When intracavitary drains are employed, certain prerequisites are necessary. If a nonsuction drain is used, its exitus should be in the most dependent position feasible to favor gravity, be grooved or stuffed to promote capillary action, and rigid enough to withstand the pinch-cock mechanism of abdominal wall pressure, which may effectively block the drainage exitus. The most popularly used drain, the plain rubber Penrose, meets none of these criteria and should not be employed for abdominal cavity drainage. In many cases the first manipulation of the Penrose drain in the postoperative period resulted in the release of a considerable amount of trapped exudate, demonstrating its inadequacy. Alternatively, the corrugated rubber drain fits the above-mentioned criteria, and has been most satisfactory.

If suction drainage is to be employed, the vacuum type is most effective. It functions independently of gravity, and is also a closed system which tends to diminish the threat of outside contamination of the wound.

DURATION OF OPERATION

The study supports the contention that increased operating time enhances the potential for postoperative wound sepsis (3, 4) (Table IX). Several mecha-

TABLE VIII

Incidence of Wound Infection with Wound Drainage

Drain	No. of wounds	No. of wounds infected	Infection rate (%)
Absent	777	62	4.6
Present	518	35	12.0

TABLE IX

Incidence of Infections by Duration of Operation

Duration (hours)	No. of wounds	No. of wounds infected	Infection rate (%)
0–2	654	35	5.4
2–3	296	19	6.4
3–4	221	20	9.0
4–5	90	15	16.6
5+	34	8	23.0
Total	1295	97	7.5

nisms may be involved, including: (1) contamination—saturation of the wound with microorganisms from primarily endogenous sources; (2) personnel fatigue, leading to breaks in technique; and (3) prolongation of anesthesia, leading to a depression of the patient's defense mechanisms.

Longer operations imply more extensive and difficult surgery in which the disease for which the surgery is being performed might enhance the potential for wound sepsis: 3 of the 8 patients who underwent operative procedures lasting longer than 5 hours had a gastrointestinal carcinoma, 2 of the patients had biliary tract carcinoma, and 1 patient was a severe alcoholic and diabetic with massive gastrointestinal hemorrhage secondary to nonmalignant disease.

MORBIDITY

Morbidity has been defined as that period in days from operation to wound healing. The range was 14 to 180 days, and the average was 24 days. It is readily apparent from the prolonged morbidity that major postoperative wound sepsis is a serious condition: for the patient, it is a threat to life, a socioeconomic dysfunction; for the hospital, it is extended bed occupancy, and intensive and extensive medical and paramedical care, not to mention the potential mortality which in this series was 20.6 percent, and in which the wound infection contributed significantly to the demise (in 17 of the 20 deaths).

In approximately one-third of the patients wound infection was not discovered after the initial surgery for 10–14 days. This delay in diagnosis may be partially explained by the common usage of so-called prophylactic antibiotics for 5 to 7 days in the immediate postoperative period, producing a masking effect on the emergence of clinical sepsis. For example, of the 46 clean-contaminated cases, 21 had received antibiotics in the immediate postoperative period, and in 14 of these clinical sepsis was not discovered for 10 to 14 days. Certainly by this time, local infection has taken a strong foothold; what may have initially been a subcutaneous abscess may have progressed to fascial necrosis, and even wound evisceration, contributing markedly to the extended morbidity. The use of so-called prophylactic antibiotics is considered especially hazardous, not only for the masking of clinical sepsis, but also because of toxicity and the threat of inducing suprainfection through emergence of more virulent organisms by adaptation.

LOCAL WOUND MANAGEMENT

Local wound management was found wanting in 19 of the cases. Local wound care requires opening of the incision for the length of the undermining, and if necessary the entire incision, to eliminate overhanging edges which contribute to the maintenance of the infection. All suture material exposed should be removed and cultures taken for aerobic and anaerobic organisms. In conjunction with debridement, wound irrigation with topical agents is recommended, but not the commonly used antibiotic solutions such as ½% neomycin, which we have found not as efficacious as a topical antiseptic, such as modified Dakin's solution. The latter is not only germicidal, but dissolves necrotic tissue as well. Its bacteriocidal spectrum is complete, with no cross sensitization, and no resistance emerging. Moreover, its cost in comparison to topical antibiotic solutions is quite low. For example, ½% neomycin solution at this hospital costs two dollars and 80 cents per gallon, while the modified Dakin's solution costs only 25 cents per gallon.

ADJUNCTIVE CHEMOTHERAPY

When the wound has been adequately drained and debrided, the use of adjunctive chemotherapy should depend upon clinical evaluation of the patient. If there is defervescence and no sign of systemic toxicity, there is no need for chemotherapy; if these conditions do not ensue, then chemotherapy should be instituted. To select an appropriate antibiotic an educated guess must be made, as culture and sensitivity reports may take several days. A gram stain of the exudate should be made, which may suffice to establish the presence of such microorganisms as staphylococci, streptococci, clostridia, and gram-negative rods. Knowledge of the location of initial surgery and the organisms most responsible for wound infection in one's particular hospital related to that type of surgery, would suggest the most likely pathogen involved. Knowledge of the spectrum and activity of the more commonly available antibiotics in the particular institution would then determine the antibiotic to be used. In this hospital, an annual survey of the bacterial population of infected wounds is made, together with the sensitivities of these microorganisms to the antibiotics available. This is of considerable aid in the initial selection of the chemotherapeutic agent. Well over half of the pathogens in this study were of the coliform aerogenes group; thus, the most likely pathogen can be deduced, and the appropriate chemotherapeutic agent instituted, modified only by lack of clinical response, or when the particular culture and sensitivity reports reveal an inconsistency. If adequate local wound management with or without adjunctive chemotherapy does not result in satisfactory clinical improvement, a vigorous search for occult sepsis must be made. Etiology may be such complications as intra-abdominal, pelvic, or subphrenic abscesses, or may involve more remote systems, such as the genitourinary or respiratory tracts.

Conclusion

Major wound sepsis continues to remain a serious problem to the patient, to the hospital, and to the community. Technological control of the exogenous environment has never been more complete, yet overall surgical infection rates have not been significantly reduced. To effect reduction each hospital must first know its infection incidence. This entails careful documentation of data and dissemination and discussion of the information by the hospital staff. This will lead to greater understanding of the etiology and management of wound infection, the ultimate aim, of course, being its prevention, and, at the least, a meaningful rate reduction. Emphasis must be focused more on the endogenous environment (the patient as the reservoir of his own subsequent infection), with careful screening for remote infection, and its vigorous treatment prior to elective surgery, if found.

Surveillance studies of the microbiology of the hospital environment should be performed and compared to the microbiology of the infected wounds, so that the relative importance of exogenous and endogenous controls can be continually assessed. Sensitivity studies should be periodically performed to determine the bacterial spectrum of individual antibiotics, as wide variance exists in most hospitals in terms of antibiotic efficacy and microorganism sensitivity. This will enable more judicious application of chemotherapy until culture and sensitivity reports are available. Even greater attention to these details must be enforced for those special states peculiarly susceptible to high infection risk—the aged, the diabetic, the steroid patient, the obese, and the malnourished.

Only through knowledge so afforded, disseminated, and applied will major postoperative wound sepsis be significantly reduced.

Summary

Twelve hundred ninety-five surgical procedures performed during a 12-month period were reviewed; there were 97 major wound infections, with an overall infection rate of 7.5 percent.

The endogenous environment was the major source of wound sepsis; the exogenous environment's contribution was not significant.

The presence of remote infection was characterized by an almost fivefold increase in infection rate.

Certain patient factors, including age, diabetes mellitus, the use of steroids, obesity, and malnutrition, markedly increased the infection potential.

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Spermatic Cord Tumors: A Case Report

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Ninety percent of extratesticular neoplasms found within the scrotum occur in the spermatic cord. However, tumors of the spermatic cord are relatively rare. Seventy percent are benign and most of these are lipomas. Of the malignant tumors, the great majority are mesenchymal in origin and are sarcomas. We present herein a case of a benign myxofibroma of the spermatic cord.

Case Report

N. F. (MSH #352780), a 22-year-old male, was admitted for evaluation of swelling in the left hemiscrotum, first noted by the patient 6 months prior to admission. The mass was painless and had been insidiously enlarging to its present size of approximately five inches in diameter. There was no history of antecedent scrotal trauma or inflammation; there were no genitourinary symptoms. Of significance in the history was the fact that the patient had undergone a right hydrocelectomy 4 years prior to admission. Physical examination was entirely normal, save for a swelling in the upper left hemiscrotum which was non-tender, smooth, and quite firm. Forty percent of the mass transilluminated. Below the mass and separate from it was a normal palpable testis. Laboratory findings were entirely within normal limits. Intravenous pyelography showed prompt bilateral function with normal upper collecting systems, ureters, and bladder.

Due to the position of the mass, the fact that the testis was separate from it, and that a large portion of it transilluminated, the preoperative diagnosis was spermatocele (Fig. 1).

Using a four-inch left inguinal-scrotal incision over the pubic tubercle, exploration of the scrotum was carried out. An irregular rounded lobulated mass measuring 8.5 cm in the greatest diameter was found to be arising from the spermatic cord at a point just distal to the external inguinal ring. The tumor was well encapsulated and was dissected free of surrounding structures without difficulty since no vascular pedicle was noted. The testicle was found to be normal and was left in place. The patient's postoperative course was uneventful.

Pathology (MSH #P99-9726): Encapsulated acellular myxofibroma. No evidence of malignancy microscopically. The specimen was an irregular round, moderately firm tumor, measuring 8.5 cm in diameter and weighing 187 gm. The tumor was apparently well encapsulated. On opening the tumor mass it was found to be composed predominately of a gelatinous soft myxoid material, mostly yellowish tan in color.

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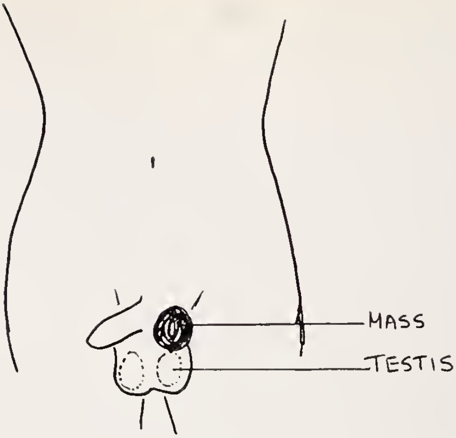

FIG. 1.

Discussion

The earliest known cord tumor was recorded by Cloquet in 1819. Thompson reported the experience of the Mayo Clinic through 1935 with these tumors. In his series there were 24 benign spermatic cord tumors and two malignant tumors. They ranged in size from 2 to 8 cm in diameter and appeared in the age range of 33 through 67 years.

Dreyfuss and Goodsett offered the following classification of cord tumors:

A) Benign
  1. Epithelial tumors
  2. Mesoblastic tumors
    a. lipoma
    b. fibroma
    c. myxoma
  3. Heterologous tumors
    a. cystic dermoid
B) Malignant
  1. Epithelial tumors
  2. Mesoblastic tumors
    a. sarcoma (lipo-, myxo-, chondrofibro-spindle cell, leiomyo-, rhabdomyo-)
  3. Heterologous tumors
    a. teratoma
  4. Metastases to the cord

Samellas presented a collective review of malignant cord tumors found in the literature up to 1964 and presented the following distribution:

Sarcoma	47	Reticulosarcoma	2
Fibrosarcoma	21	Teratoma	2
Leiomyosarcoma	11	Rhabdomyxofibrosarcoma	1
Rhabdomyosarcoma	10	Leio-osteiofibrosarcoma	1
Carcinoma	4	Angioendothelioma	1
Myxochondrosarcoma	3	Dysembryoma	1
Fibromyxosarcoma	3	Lymphosarcoma	1
Liposarcoma	3		

The Pathology of Testicular Tumors in the 1964 Supplement to the British Journal of Urology analysed 27 cases of paratesticular tumors of connective tissue and muscle. Of these, rhabdomyosarcomas and embryonic sarcomas constituted 11 cases. Fifty percent of these patients were dead within one year.

These tumors were found to occur almost exclusively in children and adolescents. It is believed that their origin is in embryonic mesenchymal tissue, rather than in adult muscle. They have a poor prognosis and tend to metastasize rapidly and widely.

Differential diagnoses to be considered in cases such as the one presented include hydrocele of the cord, spermatic granuloma, tumor of the spermatic cord, intravaginal torsion of the spermatic cord, pedunculated tumor of the tunica vaginalis (with reactive hydrocele), scrotal hernia, spermatocele (possibly multilocular), localized epididymitis, and torsion of appendix testis.

A striking diagnostic point observed in this case was the fact that this solid tumor transilluminated over 40 percent of its surface. This was probably due to its large gelatinous myxoid component, which led to a preoperative diagnosis of possible spermatocele.

Treatment

In cases of malignant tumors of the spermatic cord, radical extirpation of the scrotal contents and cord structures should be done removing as much of the proximal spermatic vessels as is possible. Since the majority are sarcomas, radical retroperitoneal lymphadenectomy is not indicated.

In this specific case, the most probable site for future involvement would be in the retroperitoneal space, especially in the pelvic region. According to Otani, this type of tumor has also been observed in the subcutaneous tissue of the thigh. The treatment for this tumor is purely surgical and should be entertained when the lesion causes symptoms similar to any benign space-occupying tumor.

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Observations on the Treatment of Strongyloidiasis with Thiabendazole in New York City

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Thiabendazole, 2-(4′-thiazolyl) benzimidazole, a broad spectrum anthelminthic introduced in 1961, has proved effective in the treatment of strongyloidiasis and some other nematode infections of man, e.g., ascariasis, enterobiasis, creeping eruption (cutaneous larva migrans), and hookworm (1–10). Most of the data concerning its value have come from countries in South America, Africa, and Asia, where human helminthiasis is common and often severe. There have been but few published reports of clinical experience with this drug in the treatment of strongyloidiasis in the United States (6, 8). It is the purpose of this paper to present the results of a study concerned with the use of thiabendazole in the treatment of this disease in New York City.

Strongyloides stercoralis, a small nematode, parasitizes the intestinal mucosa of man. Infection is most common in tropical and subtropical areas where poor sanitation results in widespread soil pollution with human feces. In the United States, strongyloidiasis has been observed among immigrants and travelers from tropical countries, natives of rural areas in southern states and Puerto Rico, and inmates of institutions for the mentally retarded (6).

The life cycle of the parasite explains many features of the epidemiology and pathogenesis of the infection. Two developmental cycles occur as follows (11):

(1) Indirect cycle: Rhabditiform (noninfective) larvae passed in feces develop into male and female adult worms in warm, moist soil. Fertilized female worms produce eggs which hatch a second generation of rhabditiform larvae. The latter develop into filariform (infective) larvae, which may repeat a free-living adult generation or infect new hosts by penetrating exposed skin or the mucous membrane of the mouth, pharynx and esophagus following ingestion. This cycle occurs where environmental conditions are optimal for the free-living existence of the species.

(2) Direct cycle: Rhabditiform larvae become filariform directly without an interposed adult generation where conditions are less favorable. Direct transformation of the larvae also occurs in the lumen of the human intestine. Infective larvae set up new developmental cycles within a host by penetrating the intestinal mucosa and perianal skin. This process, termed "autoinfection" is responsible for the marked chronicity of strongyloidiasis,

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which may last 30 years, and for occasional cases of overwhelming hyperinfection (8).

After penetrating a body surface, larvae are carried in venous blood to the lung capillaries, where they break into the alveoli and become adolescent worms. The latter ascend the bronchial tree to the glottis, are swallowed, and on reaching the duodenum develop into male and female adults. Fertilized females burrow beneath the duodenal mucosa and deposit eggs. These hatch quickly into rhabditiform larvae which enter the lumen of the bowel. In heavy infections, the jejunum, ileum, colon, and biliary and pancreatic ducts may be invaded by female worms. The eggs are rarely seen in stool specimens.

The clinical manifestations of strongyloidiasis are varied and may simulate many common diseases (12, 13). Penetration of skin by larvae produces a petechial rash, urticaria, and pruritus in the involved areas, usually the feet, legs, and buttocks. Maturing worms in the lungs cause bronchitis, bronchial asthma, and bronchopneumonia. Localization of the adult female worms in the mucosa of the duodenum and small intestine gives rise to abdominal pain, diarrhea, steatorrhea, malabsorption, and nutritional disorders (14). Chronic ulcerative colitis has been described (15). However, many individuals have no symptoms or signs of the infection.

Patients with strongyloidiasis may develop serious hyperinfection. This may occur when resistance is lowered by factors that impair the host's immune response, i.e., debilitating chronic diseases, extreme malnutrition, or treatment with adrenal cortical steroids (16–19). Since hyperinfection may be fatal, chronic strongyloidiasis, even if asymptomatic, should be treated as soon as it is diagnosed (16).

Material and Methods

Thiabendazole was given to 25 adults with strongyloidiasis at The Mount Sinai Hospital. Twenty-three attended the Parasitology Clinic of the Outpatient Department. Two were inpatients. All, except one who came from Honduras, were natives of Puerto Rico. They had resided in New York City from 6 to 15 years. Their ages varied from 12 to 59 years. Ten were female. Eight complained of attacks of abdominal pain and mild diarrhea; one of polyarthritis, and another of chronic cough and asthmatic attacks. Seventeen had no complaints.

The diagnosis was made by finding strongyloides larvae in stool specimens by direct microscopic examination of saline smears or of material obtained by formalin-ether concentration (20). Ova of Trichuris trichiura were also found in two cases, hookworm in three and Schistosoma mansoni in one. Cysts of Endamoeba histolytica, Endolimax nana, and Giardia lamblia were detected in a single case.

The drug was given in doses of 1,500 mg (20 to 35 mg/kg of body weight), in the form of chewable tablets, on 2 successive days. This was one-half the dosage that has been recommended as standard (21). Because dizziness and drowsiness may occur a few hours after taking the drug, it was given shortly before retiring. Blood counts and urinalyses were performed before treatment was started, and were repeated within a week after completion of therapy. Stool specimens were reexamined for larvae within a week after therapy and at various intervals thereafter, depending on how often patients kept their subsequent appointments at the clinic.

Results

In 24 cases, strongyloides larvae disappeared from stool specimens within a week after a course of treatment. No relapses were observed in 5 of these cases followed for 12 to 30 months, and in 19 for 2 to 11 months. Eleven to 14 post-treatment stool examinations were performed in 3 cases, 5 in 3, 4 in 1, 3 in 4, and 2 in 14.

In a single case, thiabendazole failed to eliminate the parasite despite weekly courses of treatment for 3 weeks. However, in this case, the number of larvae found in stool specimens decreased from 464 per cover slip to 1–2 per cover slip. The patient was a 44-year-old Puerto Rican woman who, in addition to strongyloidiasis, had a chronic pancytopenia of unknown etiology which was being treated with 15 mg of prednisone daily. Among symptomatic patients, abdominal distress and diarrhea present before therapy disappeared and did not recur. Blood eosinophilia (5–11 percent) present in every case, decreased within a month after treatment. In two cases, ova of Trichuris trichiura were still found after therapy; hookworm ova were found in one. Schistosoma mansoni ova and cysts of Endamoeba histolytica and Endolimax nana were also still present after treatment.

Mild side effects were noted after administration of the drug. Three patients complained of nausea, and one of vertigo, for a few hours after each dose. One patient attributed a week of anorexia to the drug; another noted that his urine had an unusual chemical odor on the day after treatment.

Discussion

Our findings confirm published observations that thiabendazole is excellent therapy for strongyloidiasis (2, 4, 6, 8). In our cases, a single 1,500 mg dose (20 to 35 mg/kg of body weight), on 2 successive days, cured 96 percent. Although this was half the usually recommended dose, the results compared favorably with those obtained with larger doses by others (4, 6, 8, 21). Franz (2) had reported cures with a single 25 mg/kg dose for 2 days in 100 percent of 28 Africans, and regarded it as the one of choice because of its effectiveness and the low incidence of side effects. Our only known treatment failure was a patient who was receiving prednisone for chronic pancytopenia while being treated with thiabendazole. In this case, it is likely that prednisone, an immunosuppressive agent, prevented cure of strongyloidiasis. This drug has been known to increase the severity of strongyloides infections (18, 19).

No side effects were noted by us in 20 cases (80 percent). We believe that this was due to the small doses we used, and to the fact that the drug was given at bedtime. Side effects of thiabendazole are dose dependent. They are frequent among patients given 50 or more mg/kg of body weight per day (9). Nausea, dizziness, and drowsiness have been the most common side effects (2). Severe tinnitus, collapse, fever, chills, skin rash, urticaria, and angioneurotic edema may follow the use of this drug. Hypoglycemia, rising levels of serum transaminase, and cephalin flocculation have also been noted as a result of therapy (21).

It is our belief that thiabendazole is the drug of choice for the treatment of strongyloidiasis. Another effective drug, dithiazanine iodide, must be given for 21 days (22). It frequently causes severe nausea and vomiting, and sudden death may follow its use (23). Pyrvinium pamoate, also a very effective drug, is well tolerated and safe (24). However, it must be given for at least 7 days (25). Although it may cause nausea and vomiting, its side effects are negligible in most cases (24, 25).

Summary

Twenty-five patients with chronic strongyloidiasis were treated with 1,500 mg of thiabendazole on each of 2 successive days. Although this was one-half the usually recommended dosage, 96 percent were apparently cured. One treatment failure occurred in a patient with strongyloidiasis also being treated with prednisone for chronic pancytopenia. Mild nausea and vertigo occurred a few hours after the administration of thiabendazole in a few patients. In view of its effectiveness, and the low incidence of side effects, thiabendazole is affirmed to be the drug of choice in the treatment of strongyloidiasis.

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Blood Count and Throat Culture in Pediatric Practice:

The Use of Sodium Nafcillin

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The purpose of this paper is to reemphasize that the diagnosis of a bacterial throat infection can only be made with a culture.

Bacterial and viral infections cannot be differentiated with certainty on the basis of the white blood count which, in any case, leaves the bacterium or virus unidentified. Our study confirmed once more that a culture can identify an etiologic bacterium at the time of infection. Antibody titers can be obtained to determine a viral etiology retrospectively.

Scope and Procedure

Between February and April 1965, blood counts and bacteriologic cultures were obtained on 109 children who had respiratory tract infections. Each patient was examined in the office or at home and a tentative clinical diagnosis was made. The throat was swabbed with a sterile cotton applicator and immediately implanted on a sheep red blood cell agar plate (Steri-Lab, Hastings, New York). The implant was then spread with a wire loop, and the culture placed in an incubator at 37°C. Beta hemolysis could be recognized in 12–24 hours. The hemolyzing colony was then further checked by reimplantation in the presence of a bacitracin disc (Case Laboratories, Chicago, Illinois); if the disc inhibits the growth of the microorganism there is a 98 percent certainty that it is a Group A hemolytic streptococcus (1, 2). When alpha hemolysis was noted, an optochin disc (Case Laboratories, Chicago, Illinois) was used to differentiate between pneumococci and alpha hemolytic streptococci (3). Both above identifications required no more than 24 hours. Further bacteriologic differentiation becomes more difficult and the culture was forwarded to a laboratory in case this was found necessary.

In an attempt to find some features differentiating bacterial from viral infection, the blood counts were analyzed in various ways.

Treatment was begun with a suspension of sodium nafcillin Unipen® (Wyeth Laboratories) in a dosage of 25 to 50 mg/kg/day, administered in four doses at 6-hour intervals. If the patient vomited or seemed severely ill, the initial dose was given intramuscularly. Treatment, if tolerated, was continued until the child was seen again—usually between 5 and 10 days after onset of illness. At that time, urinalysis was performed and a follow-up culture was obtained. All Group A beta hemolytic streptococcal infections were treated for at least 10 days.

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Results

BLOOD COUNTS

The incidence of leucocytosis and the frequency of a "shift to the left" (nonsegmented neutrophils of 6 percent or more) are shown in Table I. Also shown are the bacterial relationships to total nonsegmented neutrophil counts of more than 1500 and more than 500. None of these methods, using values differing from Wintrobe's normal blood count (4), definitely indicated whether a bacterium or a virus was the etiologic organism. Of interest, however, is the relative frequency of neutrophil counts greater than 1500/mm^3 in comparing the etiologic organisms (Table II).

BACTERIOLOGIC CULTURES

The bacteria and their relationships to diagnosed illnesses are shown in Table III. When no etiologic microorganism could be identified, the result was listed under "unknown." Beta hemolytic streptococci were found in more than one-third of the cultures.

CLINICAL RESPONSE

Despite the persistence of bacteria in a few instances described below, sodium nafcillin was effective in the infections shown in Table I. Signs and symptoms remitted within 24 hours in cases of streptococcal and pneumo-

TABLE I

Relationship of White Blood Count to Identified Bacteria

	Strep*	Pneumo†	Staph‡	Unknown§
Number of patients‖	36	26	13	38
WBC of more than 12,000/mm^3	23	20	9	14
Nonsegmented neutrophils				
6 percent or more	31	20	8	24
1500 or more	18	9	3	6
500 or more	31	19	8	27

* Beta hemolytic, Group A streptococcus (1, 2).
† Alpha hemolytic pneumococcus (3).
‡ Beta hemolytic Staphylococcus aureus.
§ No etiologic bacterium identified.
‖ Two patients with mixed infections are listed under both microorganisms.

TABLE II

*Frequency of Neutrophil Count over 1500/mm^3**

Strep	Pneumo	Staph	Unknown
50.0%	34.6%	23.0%	15.8%

* Characterization as in footnote to Table I.

TABLE III

*Relationship of Clinical Diagnosis to Identified Bacteria**

	Strep	Pneumo	Staph	Strep + Pneumo	Staph + Pneumo	Unknown
Pharyngitis†	13	5	2	2	3	11
Tonsillitis	10‡	3	3	—	—	7
Otitis media	10	10	2	2	1	17
Rhinitis (purulent)	—	1	1	—	—	—
Laryngitis and pharyngitis	—	1	—	—	—	—
Otitis externa (abscess)	—	—	1	—	—	—
Bronchiolitis	—	—	—	—	—	1
Bronchopneumonia	1	1	—	—	—	1

* Characterization as in footnote to Table I.

† One instance of mixed streptococcal and staphylococcal infection is listed under both microorganisms.

‡ Includes two instances of beta hemolytic streptococci not Group A.

coccal infection. Most patients were well when seen after 5 days of treatment, which need not be attributed to the antimicrobial therapy. Treatment was continued for 10 days in all patients with streptococcal disease, and in those patients with fluid persisting in the middle ear.

SIDE EFFECTS

Twenty-four children disliked the flavor of the medication and three refused to take it. Two children professed to liking it when it was offered on a subsequent occasion. Thirty-six patients developed loose, frequent stools, or diarrhea. Except in two instances, this condition was controlled by the concomitant administration of a non-antibiotic antidiarrheal preparation. Nevertheless, this is considered a high incidence of an undesirable side effect.

BACTERIAL PERSISTENCE

Some bacteria could be recovered in cultures despite antibiotic treatment and an apparent cure of the infection. Group A streptococci were found in one patient after 6 days of treatment for otitis media originally attributed to pneumococcal infection. They were also recovered from the pharynges of two patients after 4 and 5 days, respectively, of treatment with an inadequate dosage of nafcillin, and from the throat of a fourth patient after 7 days of treatment, during which time diarrhea developed. Pneumococci persisted for 6 days in one patient from whom Group A beta hemolytic streptococci had been eliminated, and were recovered from two other patients in whom diarrhea developed after 7 and 9 days of treatment, respectively. In one patient, staphylococci persisted for 5 days, then could not be isolated during 20 days of intensive treatment, but were recovered in culture the day after treatment was terminated.

Discussion

Analysis of the blood counts in the four ways outlined did not differentiate between bacterial and viral infections. This has also been found in other studies of upper and lower respiratory tract illnesses (5–7).

The chief value of a throat culture, particularly in pediatric practice, lies in the recognition of streptococcal infection. The culture leads to suspicion in 12–24 hours, and confirmation of a beta hemolytic Group A streptococcus can be obtained within the next 24 hours. Treatment of the patient can be instituted promptly, as well as the prophylactic treatment of other members of the family. The spread of the infection among friends and schoolmates and the well-known complications of streptococcal infectious disease can be prevented (8–10). Antibiotic therapy in other types of pharyngitis is not so urgent, and usually unnecessary.

The need for a minimal 10-day treatment of streptococcal pharyngitis has been repeatedly emphasized (11, 12). Increased oral dosage of penicillin (750 mg/day) (13) leads to higher cure rates, although 1000 mg appeared no more effective than 500 mg in one study (11). Intramuscular penicillin is considered the most reliable therapy, since failure to take oral medication is well known (14).

The presence of penicillinase-producing staphylococci has been suspected of hindering the cure of streptococcal pharyngitis (15), but this interference is denied by others (16, 17). Nevertheless, whenever staphylococci and streptococci are both present, the administration of sodium nafcillin should be considered. The merits of this compound have been explored (18, 19), and its special value for staphylococcal infection has been reported (20, 21).

A study has been published since the completion of this paper which shows that sodium nafcillin is effective but not superior to penicillin for streptococcal disease, even in the relatively infrequently coexisting streptococcal-staphylococcal infection (17).

The frequent occurrence of diarrhea is an undesirable side effect of sodium nafcillin. No comparison of the frequency of diarrhea in children receiving sodium nafcillin and children receiving the correct dosage of various oral penicillin preparations has been published. Other antistreptococcal agents are less effective, but may be used (22).

Etiologic agents vary from year to year and according to the season (23); the winter months are notorious for a high incidence of respiratory infection, particularly of streptococcal origin (5). The disease pattern in the community may give the physician an important clue as to the type of disease encountered, but it is circumstantial evidence. The appearance of the throat may suggest the presence of streptococci, but can be misleading; in one report, 85 percent of cases of exudative pharyngitis or tonsillitis were nonstreptococcal (24). Therefore, throat cultures are necessary. The bacteriologic studies are easy to perform and are neither too time-consuming nor too expensive for the practicing pediatrician. Breese (25) has described the reliability of the cotton-tipped swab as a beta hemolytic streptococcus "collector." Alternative methods of collection include the use of filter paper strips (26).

The relationship of pneumococci to otitis media is well known. The frequency of streptococcal and pneumococcal isolation in otitis media, as shown in Table III, exceeded the unknown etiology in frequency during the winter months of this study, and justifies antibiotic treatment of this infection. The uselessness of antibiotic prophylaxis in acute viral respiratory disease has been established (27).

Summary

This study of 109 children with respiratory infections demonstrated that a throat culture can quickly establish the diagnosis of Group A beta hemolytic streptococcal disease. Blood counts were not helpful in differentiating bacterial from viral infections. Sodium nafcillin oral suspension was found effective in a dosage between 25 and 50 mg/kg/day for the treatment of infections caused by gram-positive bacteria.

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Inferior Vena Cava Ligation in A Patient with Congenital Ectopic Pelvic Kidney

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Thrombophlebitis of the veins of the lower extremities and pelvis is often followed by pulmonary embolization. For this life-threatening medical emergency, inferior vena cava ligation appears to be a logical approach after appropriate anticoagulant therapy has failed. However, the morbidity of the procedure cannot be neglected. Such an operation performed in a patient with a pelvic kidney creates additional serious problems. In the following paragraphs such an occurrence will be presented.

Case Report

A.D. (MSH #330054), a 36-year-old white female, was admitted to The Mount Sinai Hospital on July 18, 1966 with a temperature of 101°F and a phlebitis of the right thigh of 1 week's duration. The patient had previous thrombophlebitis of the lower extremities followed by pulmonary emboli in 1962. In 1965, because of progressive dyspnea and congestive heart failure, a tentative diagnosis of mitral stenosis and mitral insufficiency was made, and the patient underwent open cardiac surgery at another hospital. At the time of surgery no valvular disease was found and a biopsy of the myocardium revealed idiopathic myocarditis.

On physical examination, the patient appeared dyspneic. There were rales over both lung fields. Auscultation of the heart revealed a Grade III harsh systolic murmur. The abdomen was distended, and the liver was markedly enlarged. The right upper thigh was the site of an acute thrombophlebitis. Urine analysis on admission showed a specific gravity of 1.012, pH 8, 1–2 plus protein, and 8–12 WBC/HPF. Blood urea nitrogen was normal. Following admission, the patient was given heparin intravenously. After an initial temporary improvement, she developed a phlebitis of the left thigh, and an electrocardiogram on July 20, 1966 was interpreted as acute cor pulmonale (pulmonary embolization). Therefore, on July 26, 1966 an inferior vena cava plication was performed. At the time of operation, the vena cava was found to be of rather large dimensions (approximately 4 to 4½ cm in diameter). It was the surgeon's impression that the large liver had pushed down the right kidney so that the right renal vein was easily seen within the field. A serrated clip of plastic material was passed behind the vena cava and locked in place with two silk sutures. The vena cava did not seem to contain blood clots prior to this procedure. The plicating clip was placed exactly at the confluence of the two iliac veins. The vein, which was considered to be the right renal vein, was above the plicating clip.

The patient had a febrile postoperative course and her urine showed many white blood cells and red blood cells. Culture of the urine grew out Aerobacter aerogenes and B. pyocyaneous. The patient was placed on Chloromycetin®. Her temperature slowly declined and she was discharged on August 18, 1966.

While still in the hospital, some right flank pain was experienced by the patient. This got progressively worse at home and became severe during the last 2 weeks preceding her second hospital admission. The right flank pain was associated with fever up to 101°F and dysuria. The patient was therefore readmitted to the hospital, and at this time mild right costovertebral angle tenderness was elicited. There was marked suprapubic tenderness and the sensation of a deep-seated mass. The operative scar appeared well healed. Urine analysis on the second admission showed 3+ albuminuria, 4–6 RBC and 8–12 WBC/HPF.

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An intravenous urogram (Fig. 1) disclosed a normal left kidney and bladder. There was no visualization of the right kidney up to 3 hours after injection of contrast material.

Cystoscopy under local anesthesia was performed, revealing a normal bladder. The ureteral orifices were normal in size and location. There was efflux of clear urine from the left ureteral orifice, but no efflux of urine from the right ureteral orifice. The right ureter was catheterized for a length of 12 cm. No urine was obtained. A retrograde pyelogram (Fig. 2) disclosed a right pelvic kidney overlying the sacral area. The collecting system and right ureter appeared entirely normal. A 10-minute emptying film revealed no obstruction.

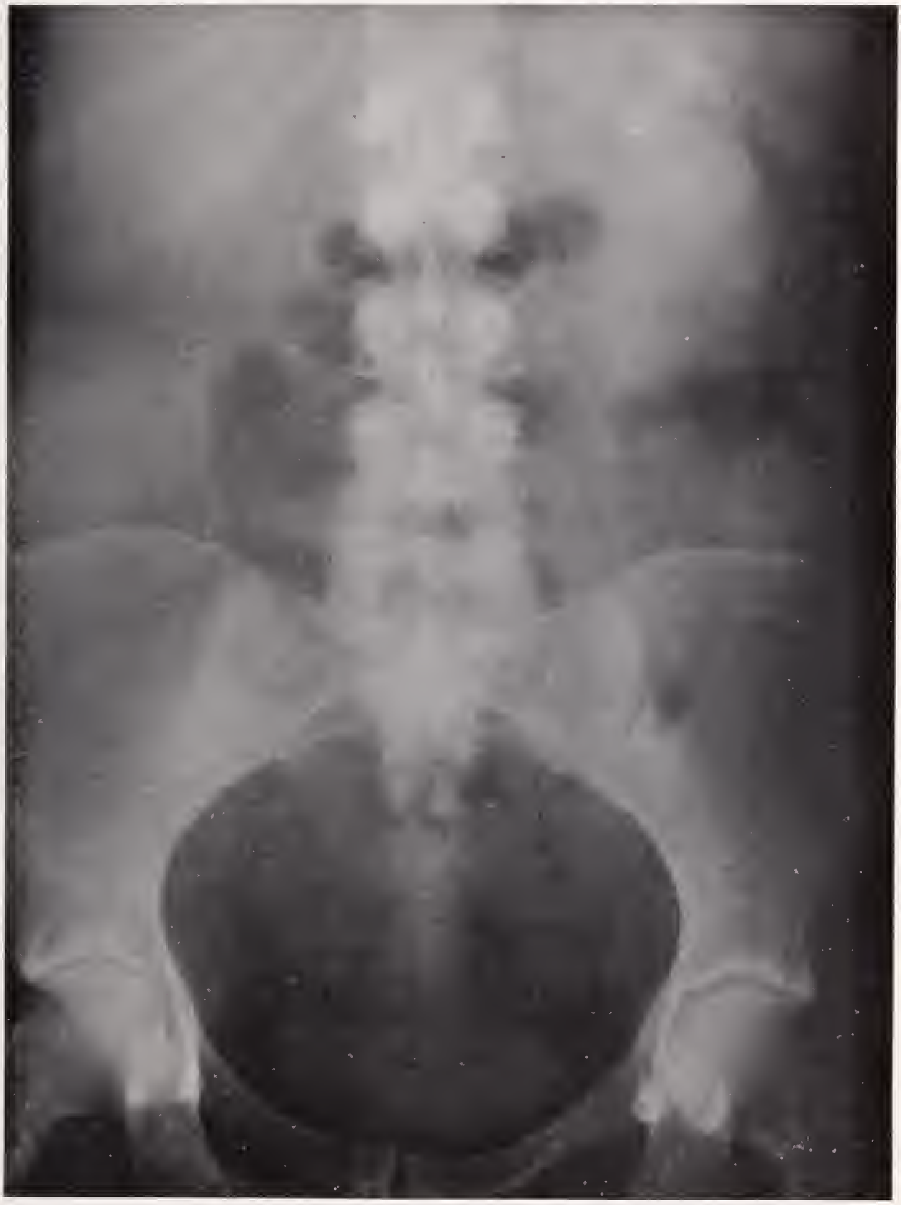

Fig. 1. Intravenous urogram showing no visualization of the right kidney.

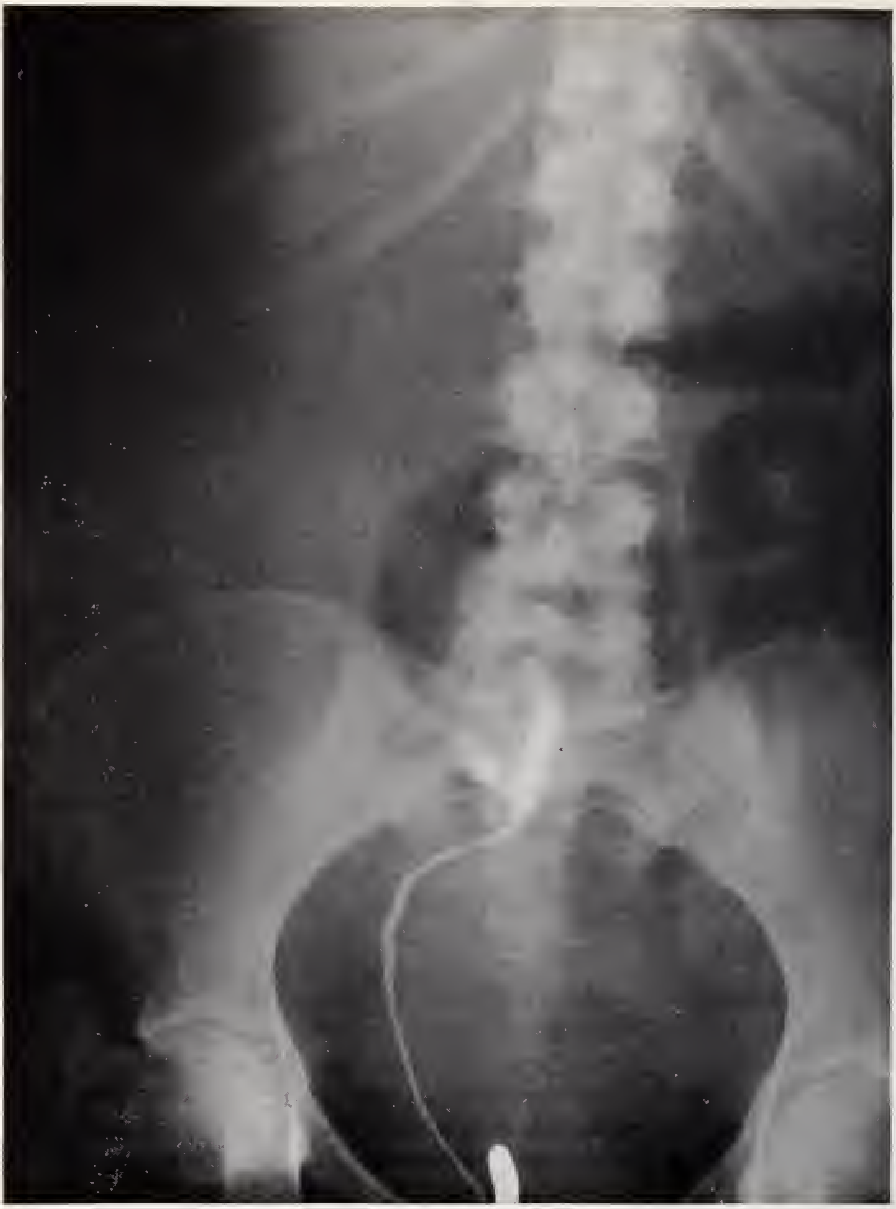

FIG. 2. Retrograde pyeloureterogram disclosing right pelvic kidney with normal collecting system and right ureter.

The patient had an uneventful postcystoscopic course. On the basis of the clinical course and x-ray studies a presumptive diagnosis of renal infarction was made, and the patient was placed on appropriate therapy.

The patient's urinary symptoms abated, but her general condition continued to deteriorate, and she developed intractable congestive heart failure and recurrent pulmonary emboli with episodes of pulmonary edema. Because of her poor general condition and prognosis, another intravenous urogram was not obtained. The patient suddenly expired on October 11, 1966; the cause of death was attributed to a pulmonary embolus. No autopsy was obtained.

Renal Embryology and Anomalies of Location of the Kidneys

Between the 7th and 8th week, ascent of the kidney takes place (1, 2). In rising from its initial low pelvic location, the previously anterior-facing pelvis turns medially. By the 5th month, the kidney reaches the normal adult position, the upper pole being at the level of the 11th rib.

The renal blood supply becomes established during the 8th and 9th week from the aorta and vena cava at the level of the second lumbar vertebra (3). Prior to this, the nephrogenic blastema gets its blood supply more caudally. The persistence of these early vessels accounts for renal ectopy, preventing the kidney from ascending to its normal position.

Congenital renal ectopia may be unilateral or bilateral. The vascular supply (4) is multiple and abnormal, and is derived from adjacent large trunks—the aorta, iliac, middle sacral, and inferior mesenteric artery. The venous return usually follows the arterial supply. In the particular case of a pelvic kidney, the arterial blood supply is even more complicated, coming from the bifurcation of the aorta, the common iliacs, the external or internal iliacs, and the middle sacral and other neighboring vessels. The veins present a similar course (5).

The incidence of congenital renal ectopia is about 1 in 800 persons when only one kidney is involved (6). According to Stevens (5), ectopic kidneys are found once in from 500 to 1,000 autopsies, true pelvic kidneys once in from 2,150 to 3,000 autopsies, and solitary pelvic kidneys once in 22,000 autopsies.

Renal Infarction

The causes of renal infarction are multiple and may involve the artery, the vein, or both (7–9).

The clinical picture of renal infarction is extremely variable. Many times it occurs without any symptoms or any urinary abnormalities. Hoxie and Coggin (10) reported in their autopsy series that in only 2 out of 205 cases had the diagnosis been made in life. Among important symptoms there is pain, which occurred in 35–40 percent of the patients, fever, which occurred in 36–67 percent of the patients, albuminuria, which occurred in about 50 percent of the patients, and microscopic hematuria, which was present in about 33 percent of the patients. Other symptoms are severe flank pain, nausea, and vomiting.

The diagnosis of renal infarction is established by x-ray studies. On the preliminary film, slight enlargement of the kidney can be seen in the acute stage. Later on, the kidney gradually decreases in size. The intravenous urogram discloses complete absence of excretion of contrast material in the case of total infarction. The retrograde pyelogram is of utmost importance. No urine will be obtained from the affected side, and there is no excretion of indigo-carmine given intravenously. The retrograde pyelogram discloses a normal pyelocalyceal architecture and no obstruction in the ureter. These studies can be confirmed by angiography, in which case total occlusion of the renal artery can be demonstrated.

Inferior Vena Cava Ligation

Venous thrombosis is not uncommon after trauma, childbirth, surgery, prolonged immobilization, and in chronic debilitating disease. In addition to the considerable morbidity of the venous thrombosis itself, there is the ever present threat of pulmonary embolization, with ensuing death of the patient. Anticoagulant therapy is designed to prevent further clotting and embolization. Inferior vena cava ligation usually prevents further pulmonary embolism. It should only be performed after anticoagulant therapy has failed (11, 12).

Many variations and anomalies of the inferior vena cava do occur. In an excellent review of the major anomalies and the lesser variations in the inferior vena cava, Edwards (13), in addition to left-sided and double vena cavas, described a preureteric cava (retrocaval ureter of the urologists) and mentioned a pelvic kidney with a renal vein emptying into the hypogastric vein. Edward's study is based on a topographical classification and on the relationships of the vessels. In the ectopic pelvic kidney, several renal veins are present entering the iliac veins on each side. In renal ectopia at the iliolumbar level, the veins empty into the iliac veins or into the veins derived from the supracardinals, namely the vena cava on the right or an abridged left cava draining to the right cava through a retroaortic intercaval anastomosis. Sometimes the left kidney, even when it is in its normal position, maintains its early venous connection in which the oblique course of the vessel is noteworthy, draining into the inferior vena cava just above the confluence of the two iliac veins.

Following inferior vena cava ligation, a collateral circulation takes over and carries blood to the heart. These collaterals are the ascending lumbar veins, the vertebral veins, the spermatic or ovarian veins, the ureteral veins, and the superficial trunk veins.

Discussion

In the case presented, because of the many organ systems involved, the responsibility for the absence of function of the right pelvic kidney cannot be determined with certainty. One can only assume that probably multiple factors played a role in it. Ectopic kidneys are prone to surgical disease such as stone formation, infection, and obstruction; it is therefore possible that this patient had a diseased right kidney prior to the vascular accident. However, no evidence for this was found on the urographic studies. Another possibility is that of an extending thrombosis from the leg veins into the vena cava taking place even before operation and causing thrombosis of the right renal vein. A third distinct possibility is arterial embolization of the right renal artery with subsequent infarction. This is possible in view of the patient's primary disease of idiopathic myocarditis, which can produce mural thrombi in the left ventricle and secondary embolization of the renal artery. Nevertheless, all this considered, it is our impression that the inferior vena cava operation had in some way interfered with the function of the right pelvic kidney. Edwards (14) pointed to the importance of the renal veins in planning vena cava ligation and stated that it is imperative to determine preoperatively the position and location of the kidneys in order to avoid ligating the inferior vena cava above the entrance of veins from an ectopic kidney. The same author (13), in 1951, emphasized the importance of the technique of caval ligation and said "that in the ligation of the inferior vena cava the best place for the tie is as close as possible to the iliac confluence in order to avoid a low left renal vein, if it exists. But even at this level, there is the distinct possibility of ligating above the veins of an ectopic kidney." The author therefore advises intravenous urography if the condition of the patient permits. Dale (11) also suggested intravenous pyelography in order to rule out the presence of a pelvic or ectopic kidney.

In view of the statistical data presented, the author wishes to emphasize the basic importance of the intravenous urogram in patients undergoing inferior vena cava ligation in order to determine preoperatively the exact location and functional status of the kidneys. Thus, injury to a kidney, and in rare instances to a solitary kidney, will be prevented.

Summary

A patient with idiopathic myocarditis developed thrombophlebitis of the lower extremities with subsequent pulmonary emboli. An inferior vena cava plication was performed following which the patient developed urinary symptoms. An intravenous urogram disclosed absence of visualization of the right kidney, while retrograde pyelography disclosed a right pelvic kidney with a normal collecting system and no obstruction. No urine was obtained from the right pelvic kidney and a presumptive diagnosis of renal infarction was made. The patient was treated conservatively and her urinary symptoms cleared, but she succumbed to her primary disease.

The author recommends preliminary intravenous urography in order to determine the location of the kidneys in all patients undergoing inferior vena cava ligation.

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Cholecystoduodenal and Cholecystocolonic Fistula:

Report of a Case and Review of the Literature

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Spontaneous internal biliary fistulae are not uncommon. They have been described as occurring between the gallbladder and each of these structures: stomach, colon, ovarian cyst, renal pelvis, bronchus, vagina, uterus, urinary bladder, and portal vein (1–8). In 1939, Wakefield (3) stated that the duodenum is the most frequently involved, and the stomach the least involved site. In a study of 41 patients by Marshall and Polk (4), 73 percent of the fistulae were between the biliary tree and the duodenum. In 37 patients fistulae were associated with primary biliary disease and a penetrating peptic ulcer was responsible in four cases.

Multiple spontaneous fistulae between the gallbladder and adjacent viscera are quite rare, however, and there are only five reported cases of a cholecystoduodenal-cholecystocolonic fistula in the world literature (6, 7). This type of fistula should be carefully defined since there is no communication between the colon and the duodenum, except through the lumen of the gallbladder. Grayson and O'Connell (8), in reporting 25 cases of benign internal biliary fistulae, were referring to a direct communication from the gallbladder to both the duodenum and the colon, a condition which was found in only 3 of their 25 cases.

Case Report

M.K., aged 73, was admitted to the Bronx Veterans Administration Hospital complaining of nausea, vomiting, and abdominal pain which he had experienced for 4 days. Past symptoms included fatty food intolerance, but there was no history of chills, jaundice, or diarrhea. Physical examination revealed an elderly white man, whose only significant finding was epigastric and right upper quadrant tenderness.

X-ray examination of the abdomen showed a round density in the right upper quadrant (Fig. 1). No air could be seen in the biliary tree. Subsequent upper gastrointestinal x-ray series suggested a small fistulous communication between the duodenum and the biliary tree (Fig. 2). Oral cholecystography with a double dose of dye did not show the gallbladder, but the films did show that the dense mass had disappeared from the right upper quadrant, and there was air in the biliary tree (Fig. 3). The large stone was subsequently seen in the pelvis on x-ray (Fig. 4), and was later extracted from the patient's rectum. It is interesting to note that retained barium from the upper gastrointestinal study refluxed into the gallbladder, demonstrating the cholecystocolonic fistula.

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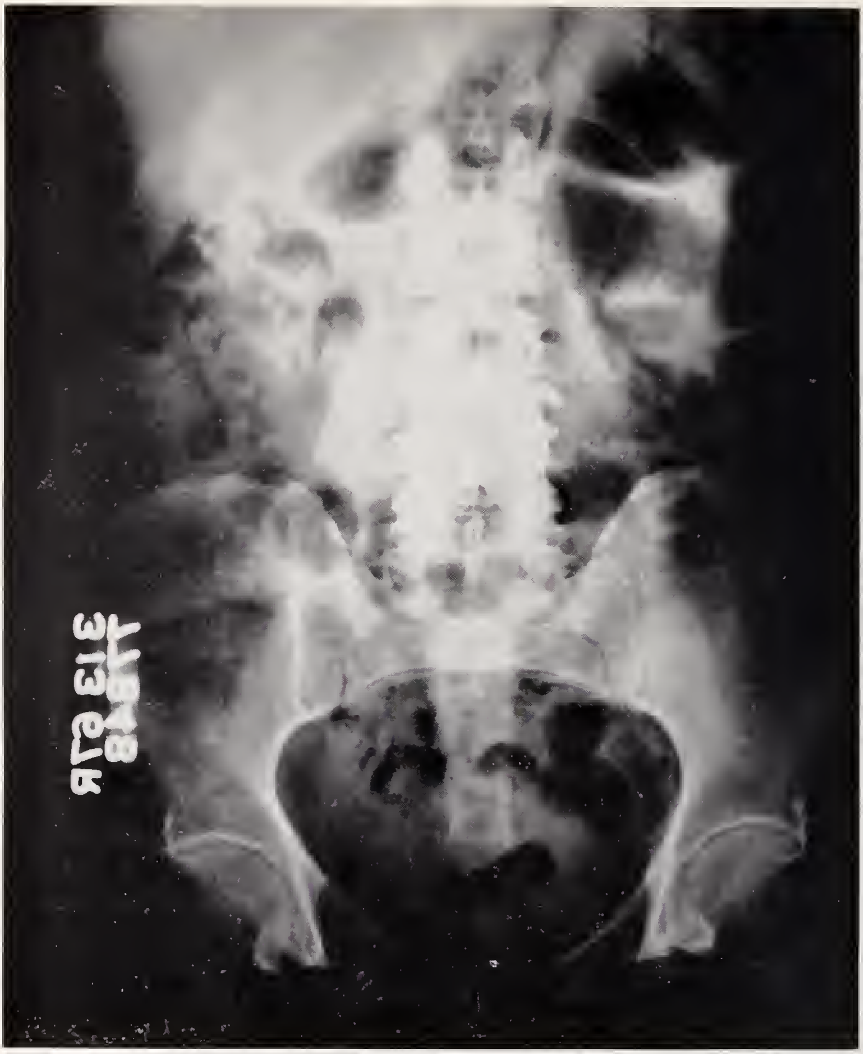

FIG. 1. Round density in right upper quadrant.

After large bowel preparation, exploration was performed. Dense adhesions in the region of the gallbladder made dissection difficult. A long-necked communication of 1 cm diameter was present between the gallbladder and the second portion of the duodenum. A separate fistulous tract of 3 cm diameter was found between the gallbladder and the colon. The common bile duct appeared normal and the small bowel was free of calculi. Cholecystectomy was performed, and the fistulous openings were closed in two layers. The postoperative course was uncomplicated and, except for the development of a small incisional hernia, the patient recovered uneventfully.

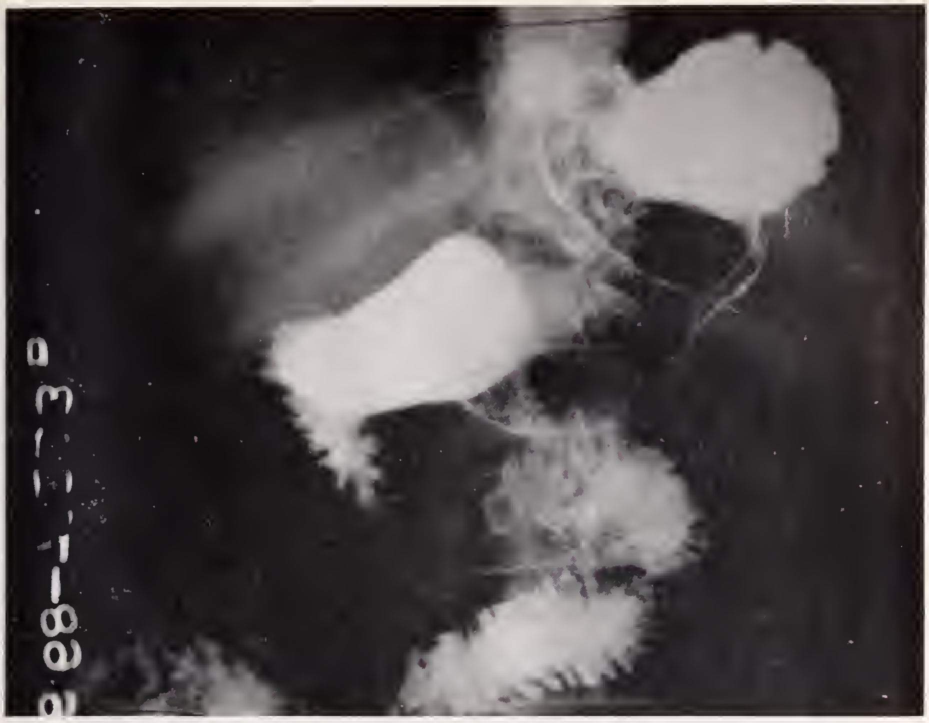

FIG. 2. Gastrointestinal series, suggesting fistula between duodenum and biliary tree.

Discussion

The proximity of the gallbladder to the colon, duodenum, and pylorus, and the mechanisms of fistulous tract formation have been discussed by Dowse and others. Fistulae of this nature are most often associated with chronic cholecystitis (4, 5). Penetration by a peptic ulcer is a much less frequent cause (9).

A review of cases at the Bronx Veterans Administration Hospital (1958–1967) revealed two patients with spontaneous internal biliary fistulae, both secondary to a penetrating peptic ulcer. One patient with a cholecystoduodenal fistula underwent a subtotal gastrectomy as treatment for peptic ulcer disease after x-ray demonstration of a choledochoduodenal fistula. A 9 year follow-up demonstrates the persistent fistula, which has remained asymptomatic.

Table I modified from Dowse (6) suggests the more specific diagnostic features of all the recorded cases of cholecystoduodenal-cholecystocolonic fistula. Marshall and Polk (4), in presenting 41 cases of spontaneous internal biliary fistulae, found a frequent history of chronic cholecystitis. Most series suggest that the major presenting symptom is abdominal pain, usually localized to the right upper quadrant. Nausea and vomiting are common. In

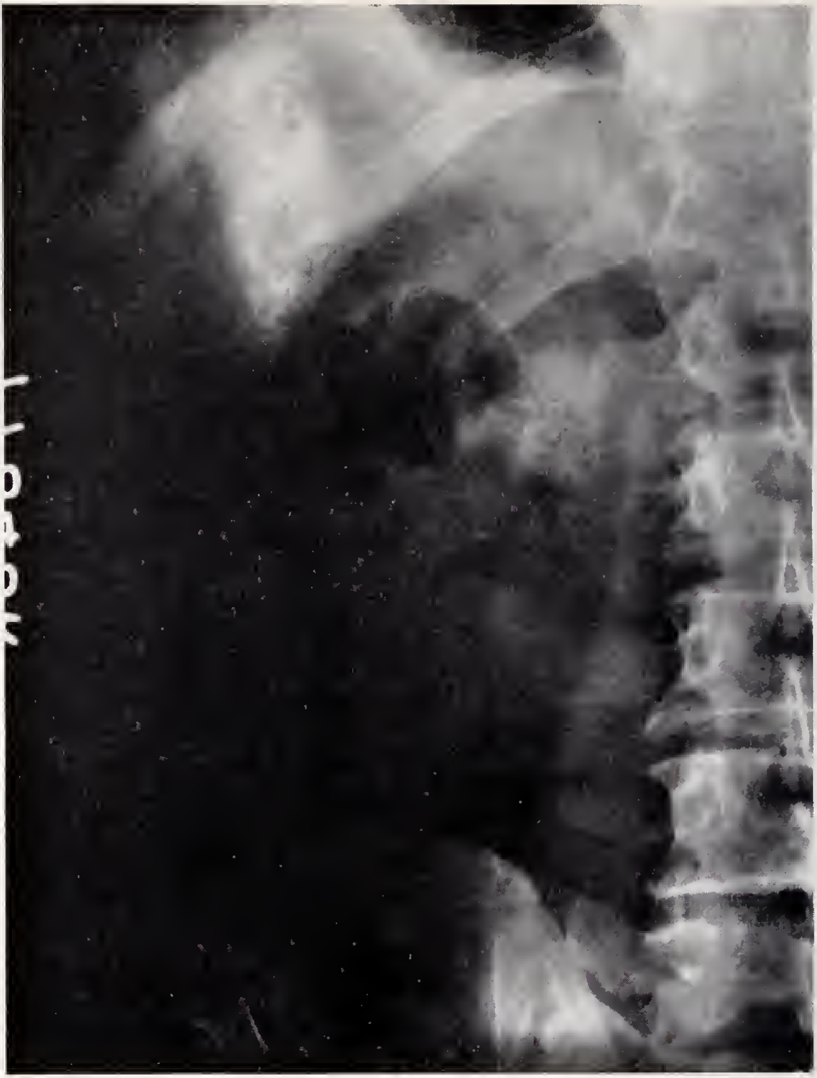

FIG. 3. Right upper quadrant mass has disappeared and there is air in biliary tree.

3 of the 6 recorded cases (although not our own), vomiting was fecal. This is strong presumptive evidence of a coloenteric fistula. Weight loss was present in 51 percent of the patients in Marshall and Polk's series.

Certain radiologic features are diagnostic, and include gallbladder calculi and the presence of air in the biliary tree. Cholecystography is of no value and only demonstrates that the gallbladder is not functioning. Barium enema studies will be the most diagnostic test when a cholecystocolonic fistula exists (9–12).

Symptoms and signs of cholangitis, jaundice, diarrhea, gastrointestinal hemorrhage, malabsorption, and gallstone ileus have been reported.

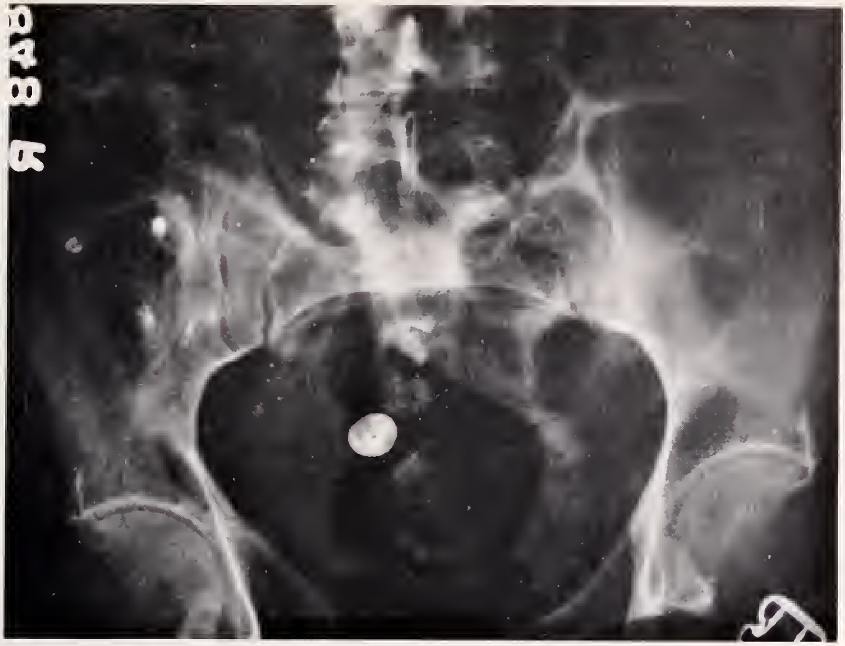

FIG. 4. Large gallstone in pelvic colon.

The association of severe bleeding with cholecystocolonic fistula has been described by several writers (12–17). The bleeding comes, possibly, from the eroded cystic artery. Massive painless rectal bleeding to shock levels has been noted. In some cases emergency surgery has been necessary, namely cholecystectomy and closure of the opening in the colon.

It has been suggested that there is a tendency for spontaneous fistulae to close (10), but a review of the cases has not confirmed this impression. The patient with an internal biliary fistula due to gallstone ulceration has a poor prognosis. The diseased gallbladder is surrounded by dense scar tissue, and stones are frequently present within the gallbaldder (56 percent) and

TABLE I

Clinical Features of Six Reported Cases of Cholecystoduodenal, Cholecystocolonic Fistulae due to Gallstones

Author	Age	Sex	Pain	Vomiting	Diarrhea	Jaundice	Diagnosis	Treatment	Result
Lovell (1947)	55	M	++	++ Fecal	—	+	Barium enema	Operation	Lived
Neville (1954)	?	M	+	+ Fecal	?	?	Barium enema	Operation	Lived
Rosenquist and Sjoberg (1955)	61	M	+	—	+++	—	Barium enema	Operation	Lived
Edmunds and Havard (1961)	68	F	+	+	—	—	Barium enema	Operation	Lived
Dowse (1963)	76	F	++	+ Fecal	+++	+	Barium meal	Operation	Lived
Present case	73	M	++	+	—	—	Barium meal	Operation	Lived

the common bile duct (29.3 percent). In addition, the fistulae rarely provide adequate drainage and are the pathways for ascending cholangitis and hepatocellular damage.

Dowse and others strongly advocate prophylactic removal of the diseased gallbladder before fistulae can form, and Marshall and Polk point out that 73 percent of their patients had symptoms for 6 months or longer.

The treatment is surgical and operation is difficult and hazardous in these patients. Supportive measures of electrolyte and fluid replacement and antibiotics should be employed. Preoperative bowel preparation is desirable. The gallbladder is usually small and contracted, and dense adhesions make identification of essential structures difficult. Surgery is directed toward the relief of present or potential obstruction by removal of the diseased gallbladder. Operative cholangiography may be helpful, and the common bile duct should be explored if indicated. Closure of the fistulous openings to the colon and duodenum, once isolated, should present no difficulty. The gastrointestinal tract should be carefully inspected for residual stones, which could produce intestinal obstruction.

The operative morbidity and mortality of patients with internal biliary fistulae are considerably higher than that of patients with uncomplicated biliary disease. Colcock and McManus (18) in reporting experiences with 1,356 cases of cholecystitis and choletithiasis, gave the operative mortality of the entire group as 1.1 percent. In contrast is the operative mortality of 23 percent reported by Dowse (7), and 12.5 percent in the series of Marshall and Polk (4) in cases with fistulae. In addition, Marshall and Polk recorded a 20 percent incidence of serious postoperative complication. In one series by Grayson and O'Connell (8), three patients with primary spontaneous internal biliary fistula did not undergo surgery, and all three died.

The prognosis of fistulae secondary to peptic ulcer disease is considerably better, and several authors suggest that surgical intervention be limited to the complications of peptic ulceration, as in their opinion these fistulae may heal on medical treatment (Dowse [7]). In reviewing our own cases, we find one patient who underwent partial gastrectomy as definitive surgery for duodenal ulceration with choledochoduodenal fistulae formation. His fistula still remains patent on x-ray studies performed during a 9-year follow-up, although it has remained asymptomatic. This casts considerable doubt as to the ability of these fistulae to heal spontaneously. Marshall and Polk (4) performed partial gastrectomy and cholecystectomy on three patients with good results.

Summary

Multiple spontaneous internal biliary fistulae are rare. A case of cholecystoduodenal-cholecystocolonic fistula due to gallstone perforation is presented. Only five other reported cases were found in a careful search of the world literature. Diagnostic clinical and radiologic findings are discussed. The treatment is always surgical, but operative morbidity and mortality are con-

siderably higher when compared to that in uncomplicated biliary disease, another indication for surgical removal of "silent" gallstones.

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Pitfalls in the Diagnosis of Trichomonas Vaginalis by the Papanicolaou Smear

GISELLA PERL, M.D.

Trichomonas vaginalis infection does not challenge the gynecologist at present as it had done for many years. Its high incidence and stubborn recurrence humiliated the most skilled consultant, although the causative agent has been known since 1836 when Donné discovered the protozoa in the discharge of a woman suffering from vaginitis.

In 1959, after Cosar and Julou reported that metronidazole (Flagyl®) is a perfect trichomonacide *in vitro* and *in vivo*, a new era started in clinical gynecology and, when treated, complete eradication of the protozoa was achievable. A great number of studies and clinical evaluations appeared in the literature and all investigators agreed with the conclusion that metronidazole was the optimal drug for the eradication of Trichomonas vaginalis both in male and female patients.

In our Vulvovaginitis Clinic we have confirmed the accepted belief in the efficacy of this drug. In the last 17 years, since the opening of our Vulvovaginitis Clinic in 1953, we have seen 6,968 new patients. The incidence of Trichomonas vaginalis infection has been 38.9 percent.

Metronidazole is a specific drug for Trichomonas vaginalis but it will not cure any other form of specific or nonspecific vaginitis. It gives a 95–98 percent cure rate, and if failures do appear, usually the wrong diagnosis has been made. Trichomonas vaginalis is the form of vaginitis where most diagnostic errors are made. Not every leukorrhea is caused by the protozoa, and even typical clinical signs and symptoms are not dependable. Because vaginal douches, creams, and jellies may interfere with the proper diagnosis of the protozoa, repeated smears and cultures should be done when in slightest doubt. A great source of common error in the diagnosis for Trichomonas vaginalis is the Papanicolaou smear. We rely constantly and too seriously on this smear in diagnosing the protozoa. Trichomonas vaginalis does not always appear in the typical pear shaped form, with its characteristic flagella. Often, it will present itself in a rounded form similar to a polymorphonuclear leukocyte. Many rounded bodies are not flagella, but in fact are merely polymorphonuclears. This rounded form is the source of daily errors. The Papanicolaou smear often is reported as "Trichomonas vaginalis infection," and treatment is instituted without further bacteriological evaluation by smear and culture methods.

Metronidazole is the final therapeutic answer to Trichomonas vaginalis; however, the indiscriminate use of the drug may cause simple or serious side effects and may destroy our faith in a dependable specific trichomonacidal drug with a high cure rate.

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In our experience we did not find any serious toxic side effects reported in the literature while taking metronidazole even for a prolonged period or in an increased amount. Gastrointestinal symptoms, such as nausea, coated tongue, dryness of mouth, anorexia, or diarrhea appeared in 4-5 percent of the patients and disappeared in a few days after completion of treatment. Except for one case, the symptoms were not severe enough to discontinue the drug.

A few cases of relative and absolute leukopenia have been reported by other investigators; however, in our series of patients—pregnant and non-pregnant, treated with metronidazole—we did not encounter any toxic hematologic side effects. Complete blood studies done before and after the 10 day treatment period showed no depression of the bone marrow. Before commencing treatment, however, a definite diagnosis of the protozoa must be made in view of the theoretic possibility of bone marrow depression.

One more side effect has to be mentioned following the use of metronidazole: the appearance of Candida albicans in the vagina. Metronidazole enhances or reactivates the growth of the latent Candida albicans, causing a new form of vaginitis with all the clinical symptoms of Monilia.

It is a great therapeutic mistake, therefore, to rely on the Papanicolaou smear alone in the diagnosis of Trichomonas vaginalis. The only accurate and reliable method is the smear and culture method, and we shouldn't institute therapy with metronidazole without the bacteriological work-up. This is a sine qua non in eradicating the protozoa.

In the past year we tried to correlate the diagnoses of Trichomonas vaginalis by the Papanicolaou smear with the smear and culture method, using Trichomonas diluent "Ortho" for the smear, and using "Kupferberg STS" media for the culture.

Our study included 769 patients. They all had Papanicolaou smears and simultaneously they all were worked up bacteriologically for Trichomonas vaginalis.

TABLE I

Trichomonas Vaginalis Findings in Papanicolaou Smear Correlated with Smear and Culture Method

Total number of patients studied	769
Number of patients positive for Trichomonas vaginalis in Papanicolaou smear	457
Number of patients positive in wet smear and STS culture	289
Papanicolaou false positives	168
Number of patients negative for Trichomonas vaginalis in Papanicolaou smear	312
Number of patients negative in wet smear and STS culture	144
Papanicolaou false negatives	168
Number of total errors in diagnosis of Papanicolaou smear correlating to wet smear and culture method	336
Percentage error (false positives plus false negatives)	43.7%

As Table I shows, of the 769 patients included in our study, 457 had *positive* reports for Trichomonas vaginalis. But on working up these 457 patients with wet smears and STS cultures, only 289 were found to have been infected with the protozoa, an error in diagnosis in 168 cases. If these 168 patients had been treated with metronidazole without any bacteriological work-up, they would have been submitted unnecessarily to treatment with a drug which cannot be used without proper indication.

In the second group of 312 patients, in which the Papanicolaou smears were reported *negative* for Trichomonas vaginalis, our bacteriological work-up found 144 cases *positive* for Trichomonas vaginalis infection. There was an error in diagnosis in 168 cases.

Had we relied exclusively on the Papanicolaou smear report for these 168 patients, we would have never treated their infection.

Summary

Our small, but well worked-up, group of 769 cases shows that we cannot rely upon Papanicolaou smear reports alone in making the diagnosis of Trichomonas vaginalis infection because as shown in Table I, the sum of false positives and false negatives in this series gives a total percentage error of 43.7%.

We urge that treatment be instituted only upon documentation of the protozoa by smear and culture.

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Clinico-pathological Conference

Pulmonary Lesions and Erythrocytosis

Edited by
FRANKLIN M. KLION, M.D.

A 39-year-old Negro male was admitted to The Mount Sinai Hospital because of shortness of breath.

He had been in good health until 6 weeks previously, when he developed pain in the right side of his chest which radiated to his back, was aggravated by deep breathing, and was accompanied by a cough and expectoration of white sputum. On two occasions he noted streaking of blood. He was treated with antibiotics, and 2 weeks later was admitted to another hospital because of aching pain on the right side of his chest and fever. An x-ray of the chest showed a right pleural effusion. He was treated with ampicillin, with defervescence of his fever and a decrease in the amount of pleural fluid. He was discharged 1 week later. A subsequent x-ray of the chest showed persistence of the right pulmonary infiltrate, and he was admitted to The Mount Sinai Hospital. He denied paroxysmal nocturnal dyspnea, edema of his ankles, or tuberculosis. He smoked 1½ packs of cigarettes per day, and drank one pint of alcohol per day.

He was a well-developed Negro male in no acute distress. The blood pressure was 130/94, pulse 76/minute, respirations 18/minute, and temperature 100°F. The breath sounds were decreased over the right axilla. No rales or wheezes were heard. The liver was palpated two fingerbreadths below the costal margin. The remainder of the physical examination was within normal limits. The hemoglobin was 17.5 gm, red blood cell count 5.7 million/mm^3, and white blood cell count 6,400/mm^3, with 62% neutrophils, 6% band forms, 1% eosinophils, 24% lymphocytes, and 7% monocytes. The blood urea nitrogen was 11 mg%, blood sugar 56 mg%, uric acid 6.4 mg%, creatinine 0.9 mg%, cholesterol 150 mg%, total serum bilirubin 1.8 mg%, direct reacting 0.8%, and alkaline phosphatase activity 80 International units. The serum albumin was 3.5 gm%, and globulin 5.9 gm%. Electrophoresis of the serum was not performed. An x-ray of the chest showed small and moderate-sized round nodules in both lung fields. An intravenous pyelogram performed showed poor visualization of the lower pole of the left kidney. A barium enema, upper gastrointestinal series, and aortogram were within normal limits. A liver scan performed with colloidal gold showed a large liver with decreased uptake of the lateral part of the right lobe.

On the 6th hospital day, the patient's temperature spiked to 103.8°F. Blood cultures were sterile. He was treated with aspirin, and defervescence resulted. A liver biopsy reported active cirrhosis. A pleural biopsy was unsuccessful. A re-

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peat hemoglobin was 19.9 gm%, and hematocrit 58%. He improved and was discharged on the 19th hospital day.

One week later he was readmitted because of recurrent shortness of breath, cough, and a loss of 12 pounds of weight. An x-ray of the chest showed a right pleural effusion and multiple nodular lesions in the left lung. The blood pressure was 128/72, pulse 84/minute, and respirations 16/minute. Physical examination revealed decreased breath sounds in the right lung. A thoracentesis yielded 2,400 cc of serosanguineous fluid containing blood and mesothelial cells. A pleural biopsy specimen showed chronic nonspecific inflammation. The hemoglobin was 23.8 gm%, red blood cell count 7.9 million/mm^3, and white blood cell count 7,500/mm^3, with 61% neutrophils, 4% band forms, 2% eosinophils, 29% lymphocytes, and 3% monocytes. The platelet count was 376,000/mm^3. Following thoracentesis he was discharged.

He was readmitted 2 weeks later because of right-sided chest pain aggravated by respiration and change of position. The respiratory rate was 22/minute. The right hemithorax was dull to percussion. The breath sounds were decreased over the entire right chest, and there was tenderness over the right upper quadrant of the abdomen. The liver was palpated seven fingerbreadths below the costal margin, and there was moderate pitting edema of both ankles. An x-ray of the chest revealed a large, right-sided pleural effusion, with multiple nodular densities seen in the left lung. Two thousand cc of serosanguineous fluid was removed by thoracentesis. A cell block showed mesothelial cells and blood. On the following day, 30 cc of Lipiodol was instilled into the right chest. A tuberculin test was positive. The hemoglobin was 19 gm%, hematocrit 57%, red blood cell count 6.1 million/mm^3, reticulocyte count 4.4%, platelet count 212,000/mm^3, and white blood cell count 7,000/mm^3, with 76% neutrophils, 12% lymphocytes, and 12% monocytes. The blood urea nitrogen was 8 mg%, serum sodium 138 mEq/L, LDH 650, SGOT 180 units, alkaline phosphatase activity 100 International units, total serum bilirubin 1.8 mg%, and cholesterol 155 mg%. The serum albumin was 2.3 gm%, and globulin 4.3 gm%. He improved with bed rest, and was discharged after 10 days.

Four days later he again noted severe shortness of breath and vomiting. On readmission the blood pressure was 70/50, pulse 100/minute, respirations 10/minute, and temperature 98.6°F. He was in acute respiratory distress, with decreased breath sounds over the entire right lung and cyanosis of the nails. The liver was palpated four fingerbreadths below the costal margin. The hematocrit was 65%. He received intravenous fluids and oxygen therapy, and expired 9 hours after entry.

*Dr. H. Gilbert**: The protocol is entitled, "Pulmonary Lesions and Erythrocytosis." I will first discuss the erythrocytosis, and then its relation to this man's disease.

The first question to be answered is whether a true erythrocytosis, that is, an absolute increase in the size of the circulating red cell mass, was present. Since the red cell volume was not measured directly, we must decide on the basis of the hemoglobin and hematocrit values. When the patient was first seen, his hemoglobin was at the upper limits of normal. As he became progressively worse, a steady rise in hemoglobin and hematocrit occurred. Does an elevated hematocrit always signify an erythrocytosis? It is important to remember that the hematocrit is really an expression of the relative size of the circulating red cell mass and plasma volume, and that it is sampled from venous blood. Therefore, alterations in either red cell mass or plasma volume will affect the venous hematocrit. For example, a diminution in total plasma volume (as in dehydration), or a shift of plasma from the venous to the capillary compartment, will produce an elevated venous hematocrit even though the size of the red cell mass is not increased. This is not an absolute erythrocytosis, but rather a relative increase in the proportion of red cells as compared to plasma in the venous blood.

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In some cases, the cause of this "relative" polycythemia is obvious. However, unexplained chronic hematocrit elevations are sometimes found in middle-aged, stocky men. Based on the hematocrit value, which is moderately elevated (in the range of 55–60 percent), these subjects have an erythrocytosis, yet measurements of the red cell mass are normal. This condition is called "spurious polycythemia" or "relative polycythemia," and is not a disease of red cell production, but an alteration in the proportion of plasma and red cells in the venous blood. It is extremely important to distinguish the person with a high hematocrit and a normal red cell mass from the patient with a true increase in red cell mass. The only satisfactory way of separating these two conditions is by direct measurement of the red cell volume, using isotopically-labeled red cells. Due to the altered distribution of plasma and the abnormal relationship between red cell mass and plasma volume, calculation of the red cell mass from the measured plasma volume and the venous hematocrit are inaccurate, and give falsely high red cell values, particularly in spurious polycythemia.

Not only is it possible to have a high hematocrit in the presence of a normal red cell mass but, conversely, it is possible to have an erythrocytosis in the presence of a normal hematocrit. This may occur if the erythrocytosis is accompanied by cirrhosis or congestive heart failure, where there is an expansion of the plasma volume. Due to the hydremia, the hematocrit will be normal even though both the red cell mass and plasma volume are increased. In this situation, only a direct red cell mass measurement will reveal the presence of an absolute erythrocytosis.

It is entirely possible that in this patient who had cirrhosis, and may have had an expanded plasma volume, the hemoglobin of 17.5 gm% may have been a reflection of an erythrocytosis. Since this man's hemoglobin and hematocrit continued to rise and reached levels higher than those usually seen in dehydration or spurious polycythemia, an absolute increase in red cell mass was probably present, and I will accept the diagnosis of erythrocytosis.

Once a true erythrocytosis has been documented, the next important question is whether the erythrocytosis is isolated or is accompanied by proliferation of other hematic cells. If there is a generalized increase in hematopoietic activity, we are dealing with polycythemia vera. This is one of a group of myeloproliferative syndromes which are characterized by a growth disturbance of the mesenchymal precursor cell that gives rise to the hematic cell populations (erythroid, granulocytic, and megakaryocytic), as well as to the cells of the marrow stroma (fibroblasts, osteoblasts, and osteoclasts). In polycythemia vera, the normal mechanisms which regulate hematopoiesis are superseded, resulting in an increase of functioning hematopoietic tissue within the bone marrow, as well as a reactivation of dormant hematopoietic tissue in extramedullary sites (particularly spleen and liver).

In this patient, the normal white cell count, the absence of early granulocytic forms in the peripheral blood, the normal platelet count, and the absence of splenomegaly speak against the presence of polycythemia vera. Although hepatomegaly was present, it was probably not due to extramedullary hematopoiesis, since the spleen usually enlarges first, or at the same time as the liver in polycythemia vera.

Since there is no evidence of myeloproliferative disorder, the problem appears to be an isolated erythrocytosis. In order to understand the mechanisms that can produce an erythrocytosis, it is helpful to consider the normal control of red cell production. The function of the erythrocyte is to carry oxygen to the tissues, and the size of the circulating erythrocyte mass is determined by the oxygen requirement of the tissues. Inadequate tissue oxygenation is a stimulus to the elaboration of erythropoietin, a hormone which, in turn, stimulates the bone marrow to produce more erythrocytes. The result is a compensatory erythrocytosis. The increased oxygen-carrying capacity of polycythemia blood may not completely compensate for the hypoxia, but it does result in improved tissue oxygenation.

Can we attribute this patient's erythrocytosis to arterial oxygen unsaturation? We did have multiple nodules within the lungs. However, it is remarkable how infrequently extensive primary or metastatic carcinoma produce arterial unsaturation. When secondary erythrocytosis does occur with carcinoma, it is usually as a result of lymphangitic spread which causes an alveolar capillary block. This is unlikely in this patient, since he was neither tachypneic nor cyanotic. In all likelihood he was not hypoxic on the basis of his pulmonary disease.

Tissue hypoxia may also occur when hemoglobin has been altered so that its ability to bind or release oxygen is impaired. In the former instance (methemoglobin, carboxyhemoglobin, and sulfhemoglobin), the arterial oxygen content is reduced. In the latter case, where the affinity of the hemoglobin for oxygen is increased (hemoglobin Chesapeake, Rainier, Kempsey, Yakima, and Ypsilanti), tissue hypoxia may occur in the presence of a normal arterial oxygen content and saturation. Neither of these conditions appear to apply to this patient.

There is no reason to suspect tissue hypoxia as the driving force for the erythrocytosis in this case, and other mechanisms must be considered. There are a group of conditions in which erythropoietin is elaborated in increased amounts in the absence of generalized tissue hypoxia. Since the erythrocytosis is not compensatory, I refer to this as inappropriate erythropoietin secretion. The kidney appears to be the sentinel of tissue oxygenation, and is responsible for initiating erythropoietin elaboration. Thus, several types of renal pathology may be associated with inappropriate erythropoietin secretion and secondary erythrocytosis. Among these are renal cysts, hypernephroma, hydronephrosis, and renal artery stenosis. Other causes of inappropriate erythropoietin elaboration are cerebellar hemangioblastoma, pheochromocytoma, uterine fibroids, renal artery stenosis, excess androgen production, or exogenous androgen administration. In many of these cases, erythropoietin has been found to be elevated in the serum, urine, tumor, or cyst fluid. In other cases, an increase in erythropoietin has not been demonstrated, but the erythrocytosis has been corrected by removal of the cyst or tumor. This does not mean that the mechanism is not erythropoietin elaboration, since our current methods for measuring this hormone may not be sufficiently sensitive to detect minor, but physiologically significant, elevations.

In this case, hypernephroma must be considered as a diagnostic possibility, since the intravenous pyelogram failed to visualize the lower pole of the left kidney, and approximately 2 percent of hypernephromas are associated with erythrocytosis. Hypernephromas frequently metastasize to the lung, and may not produce signs and symptoms referable to the kidney. Fever, which was striking in this case, is also common in hypernephroma.

Another tumor to be considered that is associated with inappropriate erythropoietin elaboration is hepatoma. The incidence of erythrocytosis in this disease is about 12 percent. The patient had consumed a considerable amount of alcohol, and had hepatomegaly which increased rapidly during the course of his illness. In addition, his liver was tender, which is characteristic of hepatoma. The liver biopsy revealed active cirrhosis. Most hepatomas in this country develop in cirrhotic livers. The liver scan was abnormal, the serum bilirubin, SGOT, and LDH were slightly elevated, and the alkaline phosphatase activity was increased. The gamma globulin was high, but an electrophoretic pattern was not obtained. This might have been helpful, since several case reports have been published of hepatoma associated with dysglobulinemia. The low blood sugar is suggestive of hypoglycemia, another abnormality which has been reported in hepatoma.

In hepatoma, the usual clinical tests of liver function, may be only minimally abnormal, and none of the above abnormalities are specific for hepatoma. However, I believe that there is enough indirect evidence to support a diagnosis of hepatoma associated with inappropriate erythropoietin elaboration and secondary erythrocytosis.

Terminally, there was a sudden change in clinical condition. Any further insult to his already compromised pulmonary state could have resulted in acute respiratory failure. Possibly, he developed a pneumonia or pulmonary infarc-

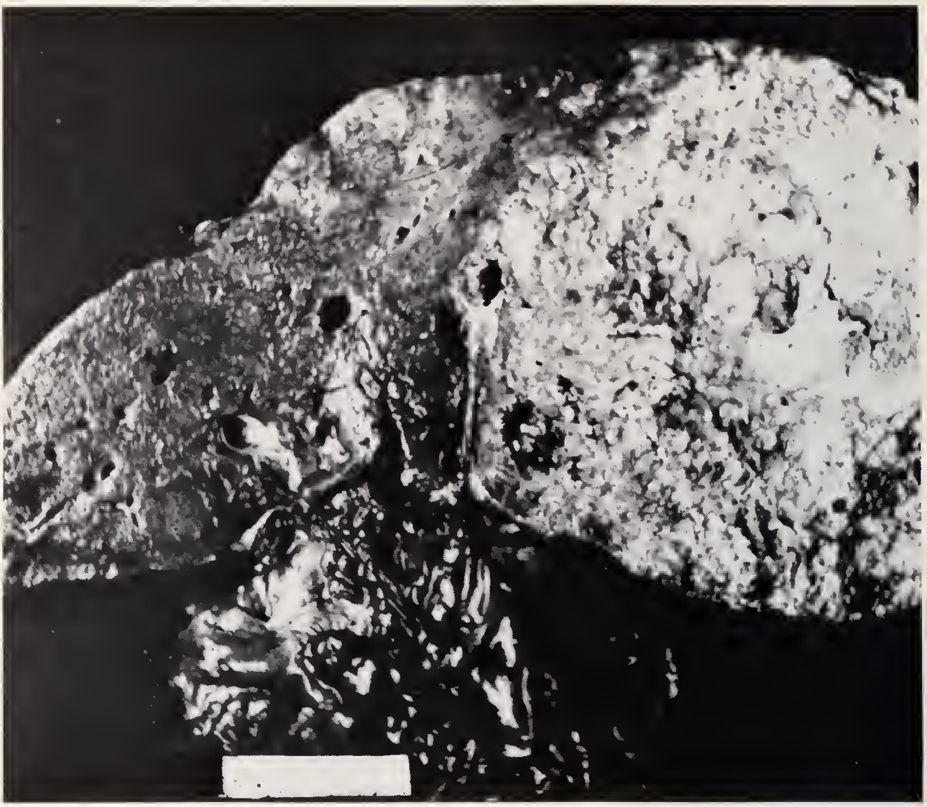

FIG. 1. Liver, showing fine nodularity on the left. The entire right lobe is replaced by a partially necrotic tumor mass.

tion. Individuals with elevated hematocrits have increased blood viscosity, and are prone to thrombotic episodes so that coronary or pulmonary infarction due to embolus or thrombosis is also very likely. An alternate explanation of his terminal episode is that he had involvement of the pericardium by tumor.

Dr. A. B. Abt:* The liver biopsy revealed an active cirrhosis, with nodules of varying size, large areas of fibrosis secondary to collapse, and bile ductular proliferation. The portal infiltrate consisted predominantly of lymphocytes and monocytes. Adjacent to the portal tracts, foamy liver cells, that appeared to be degenerating, were surrounded by polymorphonuclear leukocytes. Fat was scanty. The liver, at autopsy, showed a similar picture. Large regeneratory nodules were isolated by areas of fibrosis. Portal tracts were connected to each other and to central veins by fibrous septae. The liver cell plates were two-cells thick, indicating regeneration within the nodules. Fat in the postmortem specimen was not evenly distributed, so that it was possible that the liver biopsy did not sample one of these areas.

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The liver weighed 2,850 gm. The entire right lobe was replaced by a large, yellow-tan, necrotic tumor mass which measured some 18 cm in diameter (Fig. 1). The remainder of the hepatic parenchyma had a rather fine nodularity. The tumor mass compressed the adjacent cirrhotic parenchyma, and characteristics of hepatoma invaded the veins. Several large tumor thrombi were situated within a portal vein adjacent to the tumor mass. In other areas, large regenerative nodules compressed the hepatic veins, contributing to the post-sinusoidal hypertension. Distant from the main tumor mass were several metastatic nodules.

In spite of this man's history of heavy alcoholic intake, he did not have Laënnec's or portal cirrhosis, but what has been called a "posthepatitic cirrhosis." Microscopically, the tumor had the typical trabecular pattern of primary liver cell carcinoma (Fig. 2). The trabeculae were composed mainly of tumor cells surrounded by small endothelial lined vascular channels (Fig. 3).

The tumor was composed of large polygonal cells with very large hyperchromatic nuclei and prominent nuclei. In a few areas, there was a tendency toward acinar formation. Another characteristic of hepatocellular carcinoma is the presence of multinucleated giant cells. Since hepatoma is sometimes a sequela of hemochromatosis, a Prussian blue stain was performed. Only small amounts of iron were present within Kupffer cells.

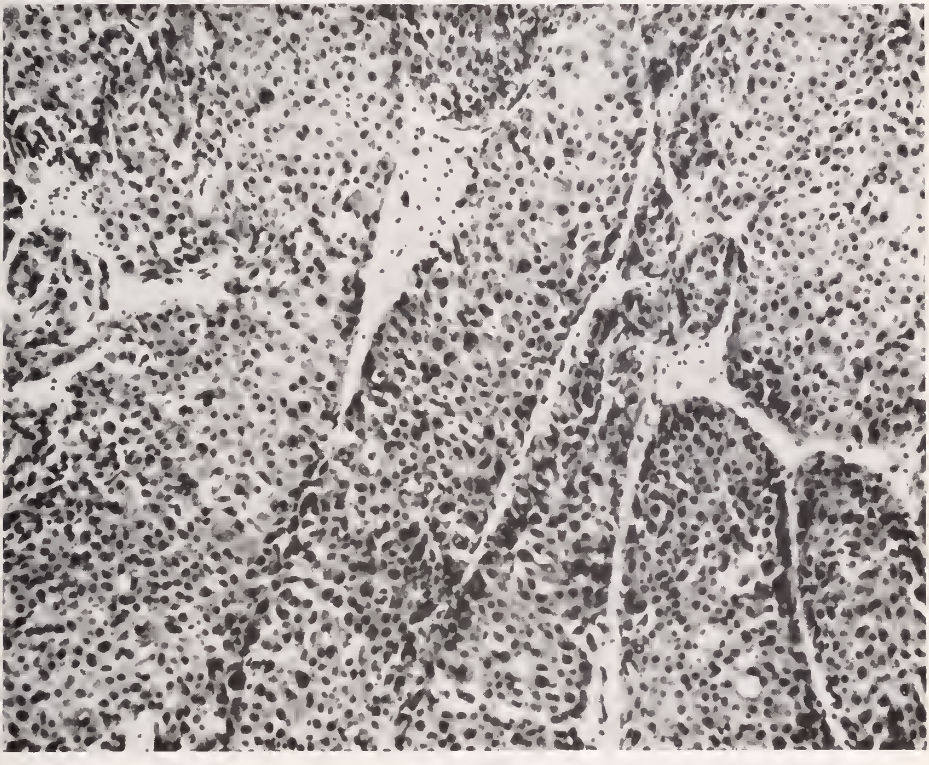

FIG. 2. The tumor is composed of trabeculae of carcinomatous cells (H & E × 100).

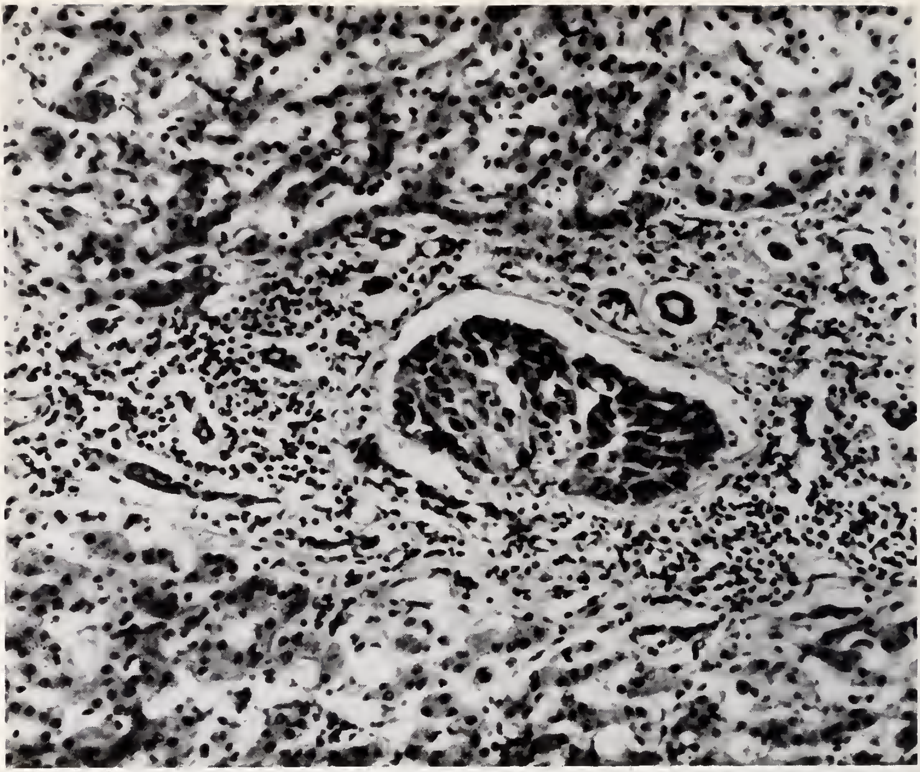

FIG. 3. A branch of the portal vein partially obstructed by a tumor embolus (H & E × 100).

This case was selected because liver cancers are unusual. Within the United States and most western countries, liver cancers are rather uncommon. In many autopsy series, they compose from one-quarter to one-third of 1 percent of all Bantu of Africa, and compose some 86 percent of all male cancers. If female cancers are included, it still composes approximately 50 percent of all tumors among the Bantus.

Hepatomas are also found with great frequency in areas of the Far East, including Indonesia, China, Thailand, and the Philippines. In these areas, the incidence is similar to that of the Bantu. The etiology of hepatomas is unknown. Many factors have been implicated, particularly nutrition. These are areas of the world where protein deficiencies are common and kwashiorkor is rampant. A few toxins have also been implicated. A toxin produced by Aspergillus flavus, known as aflatoxin, has been shown to produce tumors in rats, ducks, and trout. This is a fungus which is found not only throughout these areas, but also in the United States.

About 60 to 90 percent of all hepatomas are related to cirrhosis. The type of cirrhosis appears to make a difference. Most commonly, it is associated with posthepatitic cirrhosis or postnecrotic cirrhosis. In true nutritional, or

TABLE I

Relationship of Hepatoma to Type of Cirrhosis

Type of cirrhosis	No. of cases	Hepatoma	Percent
Posthepatitic	217	28	12.9
Nutritional	161	2	1.2
Mixed	86	2	2.3
Obstructive	71	1	1.4
Postnecrotic	69	14	20.3
Congestive	51	1	1.9
Pigmentary	27	1	3.7

From Gall, E. A.: AMA Arch Path 70, 1960.

TABLE II

Paraneoplastic syndromes associated with liver cell carcinoma

Hypoglycemia	Macroglobulinemia
Hypercalcemia	Cryofibrinogenemia
Erythrocytosis	Eosinophilia
Precocious puberty	Neutrophilia
Cushing's syndrome	Thrombocythemia
Hypercholesterolemia	Hyperlipemia

Laënnec's cirrhosis, hepatomas are uncommon, occurring in from 1 to 5 percent of the cases. There is also an increased incidence of hepatomas in pigmentary cirrhosis (Table I).

This case was also of interest because of the paraneoplastic syndromes associated with hepatocellular carcinoma. The most common ones are hypoglycemia, hypercalcemia, and erythrocytosis (Table II).

Hypoglycemia has been reported in 30 percent of cases by McFadzean, from Hong Kong. However, the incidence is much less in the United States.

Hypercalcemia has been reported infrequently. Many of the cases simulate hyperparathyroidism.

Dr. Ralph Zalusky will now discuss the erythropoietic studies performed on this patient.

*Dr. R. Zalusky**: Erythrocytosis has already been commented upon. Our current concepts concerning the erythropoietin elaboration are rapidly changing. At one time, it was felt that there was a direct relationship between the level of tissue hypoxia mediated through the glomerular apparatus of the kidney and erythropoietin production.

Recent evidence suggests that kidney releases a factor which activates a substrate present in the serum. Although a renal erythropoietic factor can be isolated from normal kidneys which, when incubated with normal serum elaborates erythropoietin, the serum factor has never been established. Whether hypoxia signals the kidney to elaboration, a factor which would then activate a substrate, is unknown. If the hepatoma is not associated with the elaboration of erythropoietin, but perhaps with substrate, then we would expect higher levels of erythropoietin.

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To assay erythropoietin, polycythemic mice are produced by keeping them in a hyperbaric chamber. Once the red cell production has been turned off, the mice are injected with test materials. In the mouse that has been completely suppressed, the Fe^{59} uptake in 48 hours is approximately 0.7 percent. However, mice that are injected with 0.05 International units of erythropoietin stimulating factor have a larger percentage uptake of the radioactive tracer. Normal human serum has no activity, and extracts from rat kidneys indicate that the renal erythropoietic factor itself has no activity.

A light mitochondrial fraction of the nontumorous portion of the liver of this patient was tested, and had no activity. When that same fraction was incubated with normal human serum to see whether or not the liver extract could generate erythropoietin in normal serum, again no activity could be demonstrated. The homogenate of a normal liver had 2 percent uptake, possibly representing very slight increase over the normal. The whole homogenate of the liver, plus the rat activator, also showed no activity. The light mitochondrial extract from the tumor alone, and the homogenate of the liver tumor showed no uptake. However, the homogenate from the liver tumor, plus the activator from rat kidney, showed 9.8 percent uptake of Fe^{59}. The patient's plasma, however, did contain erythropoietin.

We could conclude from this that if erythropoietin is really a product formed by a factor from the kidney which activates a substrate, then this patient had "substrate activity" present only in the tumor portion of the liver, and not in the nontumorous liver. However, since the patient had pulmonary complications, we do not know whether or not the erythropoietin activity in the plasma was a result of severe hypoxia activating the whole mechanism. Certainly, in the early phase of his disease, the patient's hematocrit was higher than normal, and was probably due to the hepatoma. This type of data suggests that the tumor tissue was producing an excess of a normal substrate. Since there are many syndromes associated with hepatoma, we would then have to assume that the anaplastic cell has developed the ability to make a "normal" material in abundance.

Question: Has tissue from a hepatoma with erythrocytosis been shown to have elevated erythropoietin levels when incubated with saline?

Dr. Zalusky: Only one, a saline extract of tumor from four hepatomas with erythrocytosis showed activity. Activation of a substrate by the renal factor could be explained by the trapping of blood in the tumor tissue. However, the tumor tissue alone did not activate the polycythemic mouse system.

Question: Did the metastatic lesions contain erythropoietin activity?

Dr. Zalusky: They were not tested. The kidney of this patient contained the normal amount of renal activity, which is interesting, since theoretically,

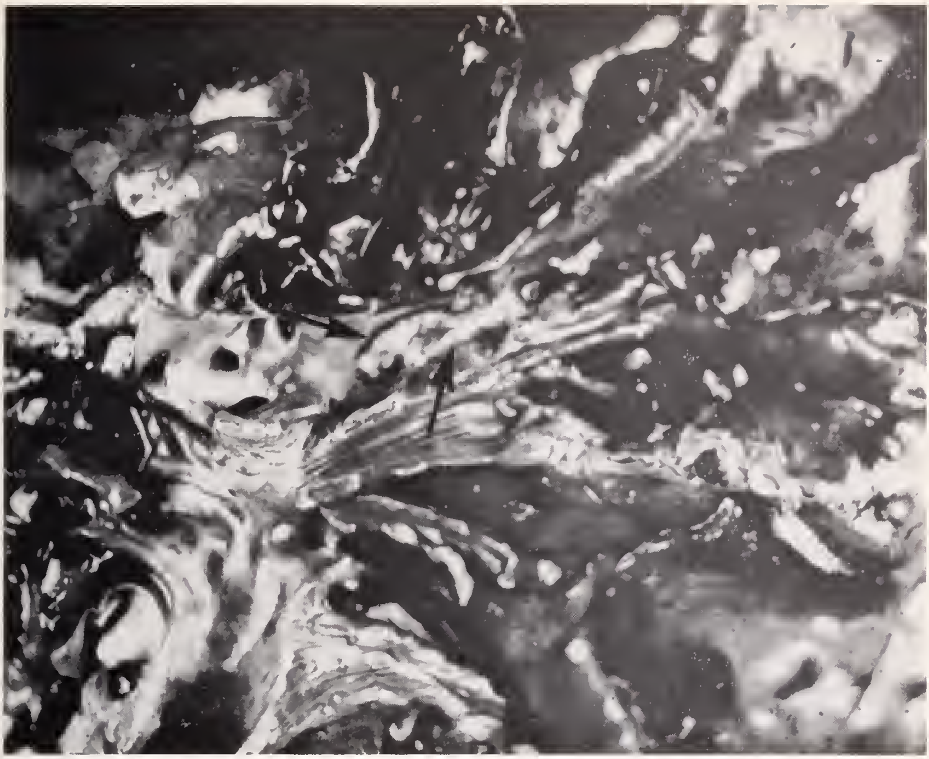

FIG. 4. Lung, showing a tumor embolus in a branch of the pulmonary artery.

erythrocytosis once established should lead to decreased amounts of REF in kidney. The superimposed hypoxia may have upset the balance.

Dr. Abt: I will now proceed with additional anatomic findings.

The esophagus was normal, except for a small erosion at the esophageal gastric junction. However, microscopically, there were large, dilated and congested esophageal varices.

The right lung weighed 800 gm, and the left 600 gm. They were heavy, and beneath the pleura were multiple white nodules of tumor. Within some of the secondary and tertiary branches of the pulmonary artery were large tumor thrombi (Fig. 4). Several of these thrombi led to pulmonary infarction. Several were green, and bile was found within the tumor cells, confirming the diagnosis of hepatoma. The source of the emboli was easily explained, since the inferior vena cava and the hepatic veins were obstructed by tumor. In addition to the tumor thrombi and infarction, there were foci of emphysema. In some areas, the tumor appeared to infiltrate through the alveolar spaces, leaving the septa intact (Fig. 5). The tumor also involved the diaphragm by direct and by vascular extension.

The bone marrow showed a moderate degree of erythroid hyperplasia, but was otherwise normal.

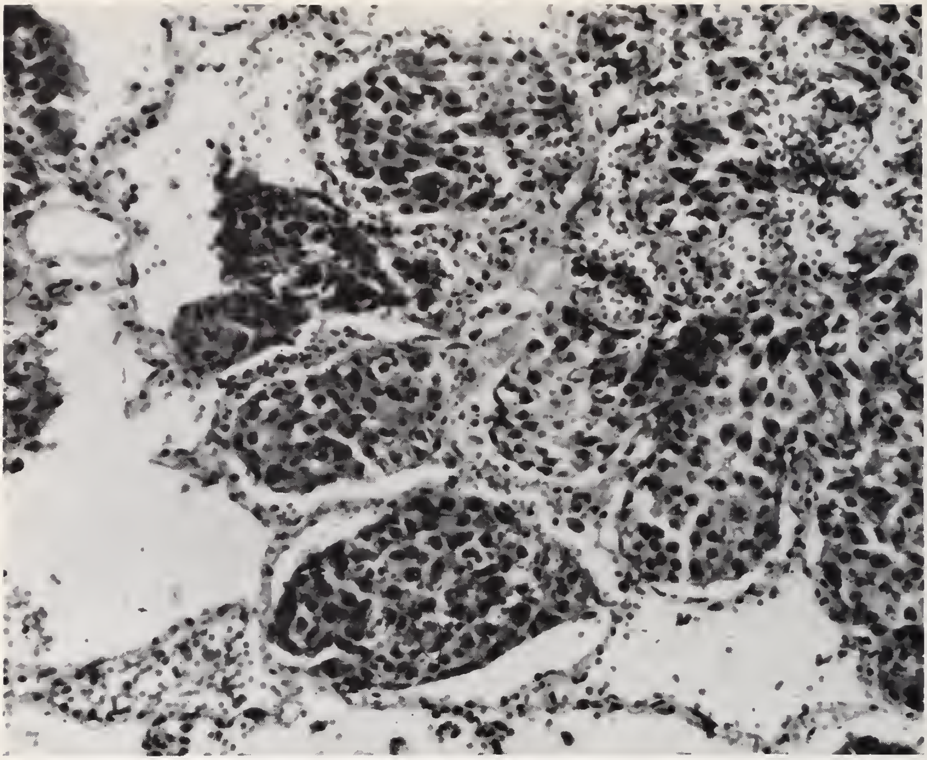

FIG. 5. Section of lung showing metastatic hepatoma invading alveolar spaces (H & E × 100).

The kidneys were normal.

The spleen was slightly enlarged, possibly due to the portal hypertension. Microscopically, there was acute congestion of the sinusoids, which was related to the recent thrombosis of the portal vein.

In summary, this patient had a hepatocellular carcinoma with extensive pulmonary emboli and pulmonary infarction. He had an irregular cirrhosis with portal hypertension and esophageal varices.

Final Diagnoses:

HEPATOCELLULAR CARCINOMA (HEPATOMA) WITH EXTENSIVE PULMONARY METASTASES AND TUMOR EMBOLI.

CIRRHOSIS, IRREGULAR.

PULMONARY INFARCTS, BILATERAL.

ESOPHAGEAL VARICES.

ERYTHROID HYPERPLASIA OF BONE MARROW, MODERATE.

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BELLA TRACHTENBERG: AN APPRECIATION

Over 50 years ago Bella Trachtenberg came to The Mount Sinai Hospital, and fortunately for us she was given a job.

With the passage of time, Bella, who had begun her association with the Hospital when Dr. S. S. Goldwater was Director, came to occupy a liaison position between Hospital Director and the Board of Trustees. She served with Dr. Joseph Turner and numerous assistant directors, many of whom now hold important positions in hospitals throughout the country. Her last close association was with Dr. Martin Steinberg, a most harmonious relationship.

But to go back, during the First World War, the members of The Mount Sinai Unit, Base Hospital No. 3, prevailed upon Bella to join with them. They had grown to depend upon her and felt their part in the War could not be best carried out without her on the job. After that stint she returned "with the troops" to the Hospital.

The Board of Trustees of The Mount Sinai Hospital now had the opportunity really to know Bella Trachtenberg. They found her to be not only reliable and fantastically efficient, but the soul of discretion as well. Pages could be filled with anecdotes from her long association with the Presidents and Officers of The Mount Sinai Hospital, but they will never be recounted, locked forever as they are within her. No leak ever came from Bella.

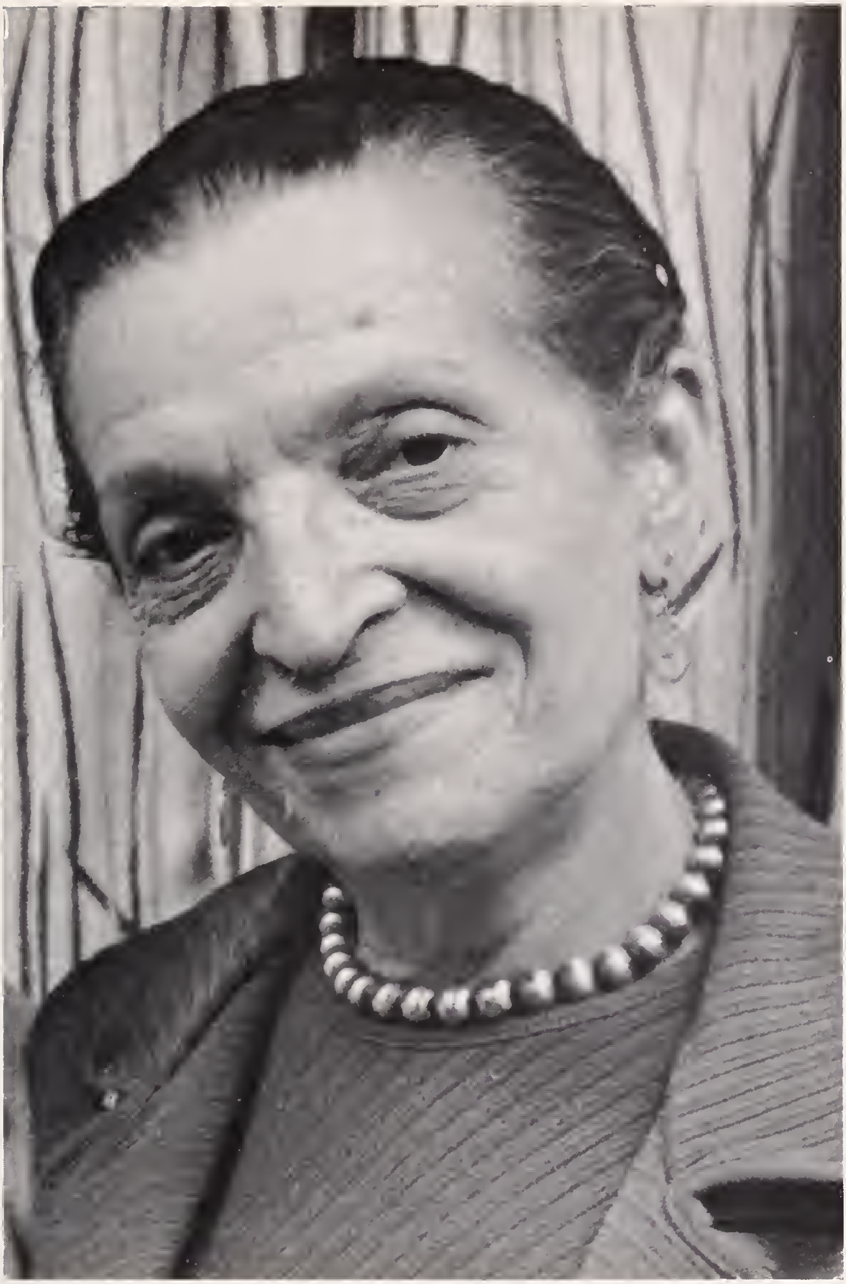
As though there were not enough hospital duties to occupy this gentle dynamo's days, she had an abiding interest in the members of the Attending, House, and Resident Staffs. She sponsored their causes courageously and continuously.

Time has never been a factor to Bella, nor the day of the week. Many times a member of the staff came in on a Sunday to see his patients and would find Bella at her desk working even into the night. When chided by the doctor, "Go home Bella. What are you doing here? Go home. Get some rest," her reply usually was, "This is the only time I can get this done. There is no one here to bother me." And how true. Actually, she loved "being bothered" and still does.

She is full of alert, youthful enthusiasms and friendliness. She attends the theatre, concerts, takes courses, travels on her vacations, and still enjoys doing a full day of work on the job.

Now that Bella has decided to fold her tent and steal away from The Mount Sinai Hospital, there will be a void which cannot really be filled by anyone. Bella is that rare, dedicated person who exudes confidence and fills one with a sense of her dependability and ability while maintaining her femininity.

She has been honored many times, by the Board of Trustees and by her many friends. She was awarded the Jacobi Medallion by The Associated Alumni of The Mount Sinai Hospital for distinguished service. But we who know Bella best, feel that what she treasures most is the warm affection of those with whom she has spent so many years of her life.



Good luck Bella. Enjoy your retirement. Shalom.

HARRY ROSENWASSER, M.D.
for the
ASSOCIATED ALUMNI OF
THE MOUNT SINAI HOSPITAL
AND SCHOOL OF MEDICINE

In Memoriam

BENJAMIN ELIASOPH, M.D.

1899–1970

Dr. Benjamin Eliasoph was born in 1899, the son of the Chief Rabbi of the Catholic city of Quebec, at that time practically a transplant of prerevolutionary France. He attended the English "public school" modeled after its British original. From there he went to Montreal where he received his bachelor's and medical degrees from the University of McGill, with its Anglo-Saxon and Scottish background. Then for almost fifty years he lived in the cosmopolitan atmosphere of Manhattan, where he died in mid-1970. This uniquely variegated background must have contributed greatly to his urbanity, flexibility, tolerance, and sense of proportion.

In 1921 Dr. Eliasoph arrived at The Mount Sinai Hospital. He was destined to spend his entire professional life there, and indeed, almost every other aspect of his life was in some way related to it. In 1926 he married Ida Endler, also a native of Quebec, and with her had two sons, James and Ira. Both of them became second generation Sinai-trained men. James Eliasoph completed a residency in urology and Ira Eliasoph one in ophthalmology. Ira Eliasoph has remained at the hospital and is a member of the ophthalmological staff.

As part of his own career, Dr. Benjamin Eliasoph completed three years on the House Staff, finishing as House Physician (the equivalent of Chief Medical Resident today). During this period, the futility of most medical treatment had led to a widespread attitude of therapeutic nihilism. Since at that time little could be done to cure disease, the young doctor learned how to treat people and how to palliate what could not be arrested. This approach became part of his being and was never abandoned, even when the steady progress of medicine in the next four decades eventually gave rise to relatively potent therapeusis.

At that time, both a tight table of organization and the custom of the country contributed to slow official hospital advancement. On the medical service, however, this constituted no bar to the utilization of the tremendous opportunities offered for the acquisition of experience, knowledge, and expertise. In turn, Dr. Eliasoph served in the Outpatient Department as an Assistant, became Chief of Clinic, Adjunct Physician, and finally Associate Physician. He was especially interested in diseases of the thyroid and was one of the early workers in the field of oxygen therapy. His basic design for the first American oxygen tent is still in general use. Concurrently, after studying under Ashchoff in Freiburg, Germany for many years, he served in the Department of Pathology, where in addition to basic training in that field, he learned the salutary lessons which the postmortem offers to the clinician.

BENJAMIN ELIASOPH, M.D.
1899–1970

Like a number of other outstanding but relatively unpublicized products of Mount Sinai, Dr. Eliasoph might best be characterized as the "Compleat Practitioner," pursuing his vocation with unremitting ardor and enthusiasm. His success as a physician was due to two factors. The first was basic training and a certain turn of mind. The second was character and personality. Keenness, flexibility of mind, and a pragmatic approach combined to help him face the diverse problems that daily confront the medical practitioner. Smugness and self-satisfaction, the procrustean approach, were not for him. While he was afflicted with that "divine discontent" which is always seeking improvement, a sober mentality prevented the premature acceptance of the untried.

In character he was a man of extraordinary integrity. He was a gentleman and also a very gentle man. This gentleness was born not of weakness but of self-knowledge and assurance, without a trace of arrogance. His patients sensed his medical competency as well as his insight, sympathy, and unobtrusive compassion. This inspired that ideal patient–physician relationship which, unfortunately, is more talked about than realized. His own concept of this relationship was very simple; the doctor was made for the patient and not vice versa. He influenced his patients without either duping or dominating them. No sacrifice of time, effort, or personal comfort was considered too great as long as it contributed to the welfare of the sick.

His breed is not extinct, but it has lately been noted to be in short supply. The prolonged and unwarranted downgrading of the Ben Eliasophs of medicine will not help to recruit replacements. It is certainly impossible, and probably undesirable, to proscribe the worship of the "Bitch Goddess Success." But the definition of success itself may, perhaps, be altered. The House of Medicine also has its many mansions. It is not necessary that they all entertain the same philosophies and premises, nor employ the same rigid criteria for the determination of quality and status.

Our new medical school will undoubtedly have its full quota of respected research scientists, clinical investigators, and scholars. Let us hope that it will also supply its full complement of Ben Eliasophs. After all, these practitioners of the Applied Science and Art of Medicine constitute a sort of final synapse. It is there that the nerve energy of intelligent medical progress is selectively applied to the individual patient—and finally acquires the name of action.

LEON GINZBURG, M.D.
for the
EDITORIAL BOARD

In Memoriam

FREDERIC DAVID ZEMAN, M.D.

1894–1970

It is with a deep and poignant sense of loss that we record the passing of Dr. Frederic D. Zeman in his 76th year.

Dr. Zeman was graduated from Columbia College summa cum laude in 1914 with honors in chemistry and German. He won election to Phi Beta Kappa and Alpha Omega Alpha when he received his medical degree from the College of Physicians and Surgeons in 1917.

Following a one-year internship in the Pathological Laboratory of the City Hospital, Dr. Zeman was commissioned a 1st Lieutenant in the Medical Corps, U.S. Army, and was assigned to the Rockefeller Institute for special training in bacteriology. With this background, he was appointed to the Empyema Commission to investigate the causes of the high mortality of empyema complicating the influenza epidemic which ravaged the army camps. He worked with Evarts Graham, Edward Dunham, and Alexis Moschcowitz, and he always looked back upon these contacts as inspirational. He became Chief of Laboratories at Camp Lee, Virginia, and later transferred to the U.S. General Hospital, Fort Benjamin Harrison, Indianapolis.

In 1919 Fred began a two-year medical internship at The Mount Sinai Hospital. His prior laboratory training qualified him for subsequent appointment as the Moses Heineman Fellow in Pathology upon graduation from the House Staff. He served with distinction in this capacity for five years. In 1925 he was appointed Adjunct Physician to the hospital, serving until 1954. During this time he acted as medical consultant on the surgical services of Doctors Neuhof and Colp. He was also physician to the Mount Sinai School of Nursing for five years. In 1935 he served as president of the Associated Alumni of The Mount Sinai Hospital.

In 1925 he first became associated with what was then known as the Home for Aged and Infirm Hebrews. From then until his death, a period spanning forty-five years, he was its medical director. With extraordinary foresight, vision, and empathy Fred Zeman transformed a domiciliary institution providing only custodial care into an accredited hospital which is recognized worldwide as a center for the scientific study of diseases affecting the aged. He was an early advocate of occupational therapy and clearly perceived the significant role of psychiatry in the management of the aged and their problems. Many worthwhile scientific studies in geriatric medicine were initiated and guided by him. Seven years ago he set up a Center of Instruction in Care of the Aged, funded by the Department of Health, Education and Welfare. Today it is the training center for professionals interested in and devoting themselves to geriatric medicine.

As Dr. Zeman's reputation in the field of gerontology spread it was only

FREDERIC DAVID ZEMAN, M.D.
1894–1970

natural that his specialized knowledge would be sought. He gave the first course in geriatric medicine for physicians under University auspices at the College of Physicians and Surgeons. He served as chairman of a committee on health, the National Council on Aging, and was a member of the Governor's Committee on Health, Medical Care and Rehabilitation. He served as Consultant for surveys of the Jewish aged in Pittsburgh, Detroit, and New Orleans. He was a member of the Geriatric Advisory Committee of the New York City Health Department. He lectured extensively throughout the United States as well as abroad in his chosen specialty.

Despite Fred's many commitments he maintained a keen interest in a broad range of paramedical subjects. One expression was to be found in his collection of historical portraits and illustrations on the aged, exhibited at the Graduate Fortnight in 1955, entitled "Problems of Aging." His preoccupation with the history of medicine won him the chairmanship of the Section of Historical Medicine at the Academy of Medicine and, with Saul Jarcho, he conducted monthly seminars on the History of Medicine at Mount Sinai from 1946 to 1951. He was also Chairman of the section on Historical Medicine of the New York State Medical Society as well as being a member of its Subcommittee on Geriatrics.

He published a series of papers entitled "Life's Later Years" which appeared serially in the *Journal of The Mount Sinai Hospital.* Death interrupted their publication in monographic form.

In 1946 he imaginatively conceived the idea of "Friends of the Rare Book Room" of the Academy of Medicine and collaborated with Dr. Alfred Hellman in its official founding. Fred's avid interest and active support of this venture were largely responsible for its steady and continuous growth. *The Academy Bookman* was wholly his brainchild and he served as editor from 1948 to 1954.

His literary interests and talents were utilized by various publications. He was a member of the editorial board of the *Journal of The Mount Sinai Hospital* from 1946 to 1948, as well as a member of the editorial boards of *New York Medicine* and the *New York State Journal of Medicine.* He evinced his interest in organized medicine by serving as Censor of the New York County Medical Society.

Fred traveled extensively, always seeking to further his knowledge in those fields of his special interests while visiting the libraries and museums of Europe and the Far East.

Dr. Frederic Zeman was a Diplomate of the American Board of Internal Medicine and a Fellow of the American College of Physicians.

Dr. Zeman enjoyed a large practice and was proud to be considered a "clinician" in the broadest sense—one whose prime function was to manage a sick person with the ultimate purpose of alleviating the total effect of his illness. His reputation in gerontology made him a sought-after consultant. His patients were devoted to him. Of him, one of those patients said, "He listened with his heart." How true those words, so simply yet movingly stated, for that was indeed the unique life force of Fred Zeman—"listening with his heart." His warm compassion, his great good cheer, his unfailing humor—all were evidenced to the very end of his days. Despite infirmities due to a crippling arthritis, he carried on his practice and his hospital obligations with unquenchable enthusiasm and dedicated effort. He was especially fortunate to have had at his side his wife, Madeleine, who shared his enthusiasm and helped make possible his many achievements through her own self-effacing dedication to his comfort, his interests, and his aspirations. To her and to their daughter, Carol, we offer deepest sympathy.

Fred will be long remembered by patients, friends, and colleagues.

PERCY KLINGENSTEIN, M.D.
for the
EDITORIAL BOARD

The End and the Beginning*

ROBERT J. GLASER, M.D.†

For a number of reasons, I deem it a great pleasure and privilege to have been invited to speak at this exercise, the First Commencement of The Mount Sinai School of Medicine. I am appreciative of the honor implicit in being asked to participate in what is in fact an historic occasion. But more particularly, the opportunity to be here today is especially meaningful to me because I was fortunate enough to serve as a consultant to the Board of Trustees of The Mount Sinai Hospital when the planning of the School was going forward. In association with a group of physicians, scientists and laymen, I attended a number of meetings here; like all my fellow consultants, I gained tremendous respect and admiration for the lay and professional leadership of The Mount Sinai Hospital. Although far too many of both the Board and Staff were involved for me to try to name them all, I particularly want to pay tribute to the distinguished Chairman of the Hospital and Medical School Board, Mr. Gustave Levy, and to the Vice Chairman, Mr. Milton Steinbach. I saw first-hand the prodigious energy, devotion, and leadership that both of these men exhibited in bringing this superb enterprise into being. As somewhat of an authority on trustees, I say with all sincerity that finer, more far-sighted, and more effective trustees I have neither seen nor can I imagine. Throughout the demanding days when the school was being planned, a large segment of the professional staff worked hard in close concert with the trustees and consultants, but few would quarrel with me if I single out your distinguished Professor of Pathology and now Academic Dean, Hans Popper, whose dedication to and understanding of excellence and untiring efforts to insure it were and will continue to be of inestimable value to the school. And last, but by no means least, the fact that this school has already reached a widely respected place among the medical schools of the country constitutes impressive testimony to the exemplary leadership of your distinguished President and Dean, Dr. George James. His wise guidance has assured a happy sequel to the efforts of the School's founders. All those who have worked so diligently to bring The Mount Sinai School of Medicine into being deserve the gratitude not only of all who come here for their medical education but, in a broader sense, from the larger society, which will be benefited by the physicians who are graduated from the school.

As I thought about an appropriate text for today's talk, somehow two phrases, "The End" and "The Beginning," came into focus. I turned both over in my mind, experimented with various combinations, including "The End of the Beginning" and "The Beginning of the End." For obvious reasons I discarded the latter promptly. After due consideration, it seemed to me that "The End and the Beginning" was appropriate. Today really does mark the end and the beginning for three very major components of The Mount Sinai Medical Center.

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* Address prepared for delivery to The First Graduating Class, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029, May 27, 1970.

† Vice President, The Commonwealth Fund, New York; Vice President for Medical Affairs, Dean of the School of Medicine and Professor of Medicine, Stanford University, Stanford, California, when this paper was written.

First of all, The Mount Sinai Hospital, which is a key part of the whole venture, inevitably has taken on a new role as a result of the development of the medical school. The Mount Sinai Hospital has long been considered one of the distinguished institutions of healing, not only in the United States but indeed in the world. Since its founding by a small group of generous, concerned citizens of this city in 1852, the hospital's history has been an exemplary one, beautifully delineated in the volume *The First Hundred Years of The Mount Sinai Hospital of New York.* I commend the history to all of you, particularly to the members of the graduating class. It provides a testimonial of the selflessness and singleness of purpose that made possible the founding and the flowering of this great hospital. When one peruses the list of world leaders in medicine who have been associated with Mount Sinai over its now almost 120 years of service, he cannot help but be awed. Starting with such great pioneers as Mott, Parker, Jacobi, Gerster, and Janeway, and continuing on through with Sachs, Libman, Klemperer, Schick, Koplik, and Lewisohn, the rolls of Mount Sinai carry the names of many of the individuals who brought American medicine to the fore. The tradition of excellence, established at the outset, has been a hallmark ever since, and now finds its full expression in the superb faculty that has been brought together to direct the educational program of the new medical school.

The creation of the medical school marks the end of one era for The Mount Sinai Hospital and the beginning of another. Although graduate education has long been a concern of the professional staff and of the Board of the Hospital, the potential for the education of future doctors was not fully realized until the medical school was formally established in 1963. Now the hospital assumes its new role, broadening its function as a teaching hospital to include the education of tomorrow's doctors. As has been true since its founding, the highest standards of patient care will prevail.

The School of Medicine, young though it be, in essence marks the end of its initial developmental phase and the beginning of its larger responsibilities. The graduation of a school's first class, like the birth of one's first child, has a special significance. The members of today's class form the first roster of the medical school alumni body, and will thus occupy a very special place in the School's history. The primary object of any medical school must be the education of tomorrow's physicians, and with the certification that these young men and women have completed the requirements for the M.D. degree, the medical school fulfills for the first time its primary objective. This fulfillment, of course, will be repeated annually as succeeding generations of students receive their M.D. degrees in the years ahead. But nevertheless, for The Mount Sinai School of Medicine, today is also in fact "The End and the Beginning"—the end of a short but exciting era, and the beginning of a far longer but equally important one.

And finally, and most significantly, today represents "The End and the Beginning" for members of the Class of 1970. They have come to the end of their formal undergraduate medical education, but almost immediately they will embark on a new, exacting educational experience as house officers, and from that they will seek their ultimate careers in one or another branch of medicine. And although their education, like the education of all good physicians, will be a continuing obligation for their entire lifetime, on this day we pause to honor them for their noteworthy achievement in completing the rigorous requirements for the degree Doctor of Medicine.

It is customary in talks such as this one for the speaker to direct some lofty, philosophic, or allegedly inspirational thoughts to the graduating class. As one who lives in the real world, I am, however, fully aware of the limited usefulness of platitudes in times such as these in which we live. Consequently, I shall try not to bore you with admonitions that at best would seem irrelevant.

Rather I would like to make some comments on the contemporary medical scene, and on the opportunity that each of you has as you begin a new era in your medical careers. The whole of medicine is in fact in a new era. In the past twenty-five years, there have been astounding changes in the overall fabric of medicine and of society. These changes, which have accelerated dramatically in the past several years, have in general improved the quality of medical education and medical care. Yet in respect to the latter, we have fallen appallingly short of achieving the potential that exists.

The major factor in the genesis of the changes alluded to has been the scientific revolution in biology. The period between the end of World War II and the present has been the age of research. It probably is difficult for those of you who have just completed medical school to realize how much new knowledge has accrued in the relatively short span of your own young lifetimes. Perhaps an example or two will make the point. I entered the Harvard Medical School in 1940 and took the course in biochemistry that year. If you accept my word that the Department of Biochemistry at Harvard was then as now an outstanding one, it will interest you to know that when I went through my lecture notes recently, nowhere did I find DNA or RNA mentioned. You would be hard put to find a medical article free of those terms today, for the nucleic acid biochemists have inherited the earth!

In 1940, open heart surgery was at best a dream, penicillin was a little known experimental drug in short supply, and the term molecular biology had really not yet become part of the scientific vocabulary. If you are still unconvinced, next time you are in the New York Academy of Medicine Library, pull out a medical text that was in use in 1940 and simply compare the table of contents with that in your own textbook of medicine. You will be surprised at the number of disease entities familiar to you that were not listed in 1940. If you pursue the matter further, you will be equally impressed with the number of new diagnostic and therapeutic modalities available today that were unheard of twenty-five years ago.

But the very fact that medicine has advanced so dramatically, and that so much new knowledge has been generated, has created one of the major problems facing our society today. We have failed to update adequately our health care system. The critical need is for a system that will bridge the gap between the laboratory and the bedside, and that will be equally and readily available to everyone in our society, be he rich or poor, black or white, young or old.

In this age of instant communication, societal problems exist on a real time basis, and they are much more apparent to all of us than would have been the case a few decades earlier. No segment of our society has been more aware of unmet social needs than our young people. In medicine, it has been our students and our house officers who have especially directed attention to the defects in our non-system of health care, and who have pointed to the magnitude of these defects in the disadvantaged sectors, urban and rural. They have rightly spoken out against the dehumanization of medicine in some areas. But in their zeal they have sometimes failed to recognize that they do not have a monopoly in respect to concern. There have been devoted, selfless physicians for as long as there has been something that could be called medicine. Furthermore, we have made great progress in the battle against disease and suffering.

Historical perspective is important and the history of The Mount Sinai Hospital provides it. The wretched living conditions that existed in this city in the nineteenth century were in many ways far worse than anything that exists today. The physicians who gave Mount Sinai Hospital its great tradition of service worked tirelessly to improve the availability of health care for their less fortunate neighbors, just as today, under the leadership of Dr. James, Dr. Deuschle, and other members of this faculty, impressive efforts are being made to improve health care in the sector of the city that lies about Mount Sinai.

To some degree, we are victims of our own success. We have carried forward scientific medicine to an impressive height, but we have fallen behind in our ability to provide the highest possible level of care for many of our fellow citizens who have properly come to expect it. This is precisely why I believe the members of this class are beginning their careers as physicians at the same time medicine is perforce beginning a new era. Major efforts are at last being directed toward the design of a more effective health care system in which preventive medicine and early diagnosis and treatment will command greater emphasis.

In retrospect, one can be critical of our medical schools, and thus of deans and faculties, for having emphasized research at the expense of teaching and patient care. In fact, it was not the emphasis on research that was excessive but rather the lack of concomitant concern with the other two elements of the classical triad that can and should be criticized. Now as we move into a period in which more and more of us must and will concern ourselves with the improvement of the health care system, it would be sad indeed if we failed to maintain respect for, support of, and the past investment in biomedical research. For without research and the creation of new knowledge, the future would indeed be dim. Patient care rendered by humane, concerned, and devoted physicians will be sadly wanting if those physicians are not well grounded in modern science and are not understanding of the way in which the science of modern medicine must be blended with the art.

It will be incumbent on each of you, irrespective of his particular interest in medicine, to be equally concerned with the science as well as the art. One of the danger signs manifest in a certain segment of medical students, whose motivation and sincerity are unquestioned, is their failure to remember that in our zeal to expand the health care system, we must not sacrifice its quality.

The task before us is a difficult one, to say the least. It is far easier to decry the failure of medicine in the United States than it is to provide a sound, effective blueprint for the upgrading of our medical care system. The answer does not lie in anti-intellectualism. Nor does it lie in assigning to the medical schools all responsibility for patient care in the country. Rather, the crisis in health care that affects urban areas as well as rural ones calls for the best efforts of members of appropriate medical school departments, collaborating with colleagues from the faculties of other university disciplines, particularly engineering, business, and law, that have important contributions to make. Innovation, experimentation, and critical evaluation are all essential. But the problem cannot be solved without the investment of substantial sums of money, and most of that money will have to come from the federal government. In turn, the people of the United States, through their elected representatives, will have to reorder national priorities. We may be the richest and most affluent country in the world, but our resources are nonetheless finite. We must come to an accommodation between the allocation of funds necessary for the security of our country in a troubled world, and the allocation of resources to upgrade the delivery of medical care and to combat urban decay, racism, inadequate housing, environmental pollution, and overpopulation—the other maladies of our ailing society.

Those of us in medicine, committed as we are to the saving of human life and the alleviation of suffering, cannot rest easy when we are faced on the one hand with a substantial unmet need for medical care and on the other with a totally inadequate health care system. Many of our medical schools face financial crises, and the cost of medical education, rising constantly, places unjust burdens on those who seek a career in medicine. At a time when we should be turning out substantially more physicians than we are, our medical schools are unable to offer places to qualified applicants. Funds for expansion and for student support are not only not being increased, they are in fact being decreased.

All of these facts, painful though they be, are as familiar to you as they are to me. The real question is what we do about it. Those of you who are about to graduate from this medical school bear witness to the fact that some concerned and devoted laymen and physicians have done something about it at Mount Sinai. They have created a medical school where there was none, and they have created one of high quality. Medical education at Mount Sinai gives proper recognition to the impact of modern science, befitting the proud tradition of research that was established long before medical education became a formal program. At the same time there is admirable emphasis on community medicine and on the development of new patterns of health care. The Mount Sinai example must be emulated elsewhere, supported not only by private citizens, but by state and federal governments as well.

The road ahead will not be an easy one, and progress will require constructive activity. I underline the word constructive for an obvious reason. The mindless violence that has plagued and so damaged many of our university campuses across the country has failed to achieve anything but a worsening of the overall tensions. Protests directed toward the medical crisis, unless they are coupled with positive suggestions for new approaches, will also come to naught.

Inevitably the young men and women, here and elsewhere, who are now joining the ranks of physicians, constitute a major resource in the battle to reform and improve the health care system. Educated in medical schools where they have learned the potential of modern science, they also bring the sensitivity and the sincere concern for their fellows and for the alleviation of human problems that should enable them to make major contributions.

American medicine has had a proud record in the past half century. There is much to build upon. Here at Mount Sinai, as well as across the country, let us view the end of one era and the beginning of another as a rare opportunity to better the human condition. Many years ago, Sir William Osler, in one of his essays, reminded us "... that we are not here to get all we can out of life for ourselves, but to try to make the lives of others happier." Sir William's advice is equally as fitting today as it was when he first made it.

I congratulate all of you who have completed the requirements for the M.D. degree on your achievement. I hope that for each and every one of you the years ahead will be productive and fulfilling ones, and I wish you Godspeed.

Received for publication June 17, 1970

The Social Implications of Liver Disease*

CARROLL M. LEEVY, M.D.†

Dr. and Mrs. Stratton, Dr. Popper, Dr. James, Distinguished Guests, Ladies and Gentlemen: It is indeed a pleasure to be here on this memorable occasion. Today we witness an important step in plans to expand preventive health measures and improve delivery of medical care during the seventies. Establishment of this laboratory for study of the liver and its diseases represents the beginning of what will hopefully be a concerted effort to interrupt the recent alarming increase in morbidity and mortality from liver disease in the United States—a rise since 1960 which is greater than that from any other disease. Focus of special attention on liver disease is urgent since it is already the fourth or fifth most common cause of death in American men over 40 years of age. Escalation of both the incidence and the death rate from liver disease has occurred despite notable advances in our knowledge of the liver and its diseases. This situation has stimulated the American Association for the Study of Liver Diseases, the American Gastroenterological Association, the Digestive Disease Foundation, and others to seek governmental and private financial support for the study of liver disease equivalent to that provided currently for other disease states. This increased support is justified since in the United States economic losses resulting from cirrhosis alone exceed two billion dollars annually.

There are several explanations for public delay in recognizing the social, economic, and health threat of liver disease. First, basic scientists and clinicians interested in the liver have previously focused their attention on its biology. Little support has been made available through the National Institutes of Health and other agencies for the kind of clinical studies needed to encourage widespread local interest in liver disease, or to develop demonstration projects which have proved so successful in cancer and heart disease. A second cause of apathy has been the tendency to equate hepatic injury with excess intake of alcoholic beverages, and the failure of our social order to accept responsibility for control of alcoholism and associated liver disease. Finally, liver disease occurs primarily among the disadvantaged and deprived segment of our society which, until now, has not been the focus of attention. For these reasons, the true incidence, the role in under-employment, or even the frequency of liver disease as a cause of death were unknown.

As we dedicate this laboratory in recognition of Dr. Hans Popper's numerous contributions as an investigator, pathologist, and educator to the science and practice of hepatology, it is in the best interest of national health goals to formulate specific plans to have liver disease receive the attention it deserves and needs. This is in keeping with the honoree's dreams and hopes engendered by unique discoveries during a lifetime of work on the pathogenesis and reversibility of liver disease. Much is needed: additional private support of the type provided by the Strattons in this magnificent gesture; inclusion of liver disease among those which are covered by Regional Medical Programs and Chronic Illness Control of the United States Public Health Services; special support for clinical care of liver disease in institutions with large concentrations of patients with chronic hepatic disorders, such as municipal, county, and Veterans Administration Hospitals; and finally, special support by the National Institutes of Health to develop better techniques for the delivery of medical care to socially deprived persons with liver disease.

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* Delivered at the dedication of the Henry M. and Lillian Stratton Foundation Laboratory for the Study of Liver Disease of the Department of Pathology, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029.

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Dr. Popper, Dr. Charles Davidson, Dr. Schaffner, and I have recently summarized the views of the Steering Committee of the American Association for the Study of Liver Diseases in this area. It was pointed out that by focusing special attention on liver disease caused by alcoholism and drug addiction, it is possible to improve both the life and health of a large segment of American society.

Studies by my associates and me in the Newark area indicate the presence of a disproportionately high incidence of liver disease in the ghetto. This is attributable to problems such as alcoholism and drug addiction, which are common because of socioeconomic and psychological factors, and there is a delay in recognizing and obtaining help for acute and potentially reversible phases of liver injury because of lack of education and interest. This situation occurs in each of the urban areas of the United States populated by deprived peoples including the community adjacent to this Hospital Center. Its occurrence, although depressing, is also challenging, since it offers an opportunity simultaneously to control major social ills and devastating physical illness.

Two-thirds of chronic alcoholics hospitalized for medical complications have significant structural changes of the liver, often in the absence of clinical abnormalities. Abstinence and provision of an adequate diet have restored normal histology in fatty liver and prevented progression of cirrhosis in 60 percent of our alcoholic patients who accept the need to improve liver function. In contrast, continued alcoholism often leads to irreversible structural and functional changes of the liver which necessitate prolonged costly hospitalization. It is apparent that we can and must prevent the dire consequences of liver failure in such persons by providing appropriate medical and social rehabilitation. The drug addict who has an enormously high incidence of unsuspected liver disease should also be the target of this approach. The problem is compounded by the presence of a high virus carrier rate among addicts, constituting a major source for spread of hepatitis to other addicts and the population at large. Again, focus on treating the injured liver may be of great help in control of addiction.

Success with either the alcoholic or the drug addict with liver disease depends upon mobilizing support from all sections of the community and the ability to modify the social environment and provide gainful employment. This is usually a difficult task because of a lack of needed job skills, often coupled with undereducation. Highly motivated and specially trained persons are, therefore, needed to work with such patients to create a proper environment and the desire to control alcoholism and drug addiction. This requires recruitment and organization of those who seek an opportunity to engage in activities relevant to our greatest social needs.

Early identification of persons who have sustained liver injury is of first importance in reducing morbidity and mortality from liver disease. Technological advances now permit recognition of hepatic disease during reversible phases, and in many instances, with proper treatment, prevent development of further injury. Measurement of enzymes released from the damaged liver and evaluation of the clearance of nontoxic dyes by ear densitometry provide valuable mass screening devices. Liver biopsy obtained in those with abnormal function allows one to ascertain the type, severity, and activity of the pathologic process. The current, almost universal, acceptance of percutaneous liver biopsy is evidence of the importance of generating an interest in liver disease. Thus, clinicians used this technique at The Mount Sinai Hospital in the thirties, but it was abandoned because its risks, now demonstrated to be negligible, were felt to outweigh its benefits.

In treatment of the sick liver we have just begun an exciting new era. To date, most measures have been directed to the control of clinical manifestations of liver failure, often with great success for short periods. It is now evident that we can also directly influence the status of the diseased liver by therapeutic programs designed to protect uninjured liver cells while stimulating repair of damaged cells and production of new hepatocytes. This has been largely accomplished by interrupting etiologic factors such as alcohol or infections, and supplying specific nutrients needed for repair or replacement of liver cells.

Since available therapeutic programs do not immediately produce dramatic improvement in symptomatology or laboratory tests, their translation into practice has been particularly difficult. This is illustrated by the continuing problem of providing to patients with active liver disease the increased amount of folic acid needed for cell repair. Experimental animal and human studies indicate that unless extra folate is provided, chronic liver injury regularly leads to body depletion of this vitamin and interference with hepatic DNA synthesis and regeneration. Despite this knowledge, precedent and law have combined to prevent use of folic acid as a food supplement, and, until recently, it has not been present in commercially available multivitamin preparations widely used to treat patients with liver disease. Such practices must be corrected by combined efforts of investigators, the pharmaceutical industry, and the Food and Drug Administration to make adequate amounts of folate and other micronutrients readily available at a reasonable price to victims of liver disease.

Successful delivery of expert medical care for the large number of patients with liver disease necessitates a significant increase in manpower. This requires expansion of current training programs for those interested principally in hepatology and gastroenterology; transmission of the newest concepts to the internist, surgeon, pediatrician, and pathologist; and sponsorship of special courses to train paramedical personnel including community expediters, social workers, and physician assistants to work in the community on ambulatory aspects of treatment and rehabilitation. A multidiscopline approach is desirable, as attested by the highly productive activities of Dr. Schaffner, a clinician, investigator, and educator who has effectively served as a bridge between the Departments of Medicine and Pathology in this Medical School and Hospital.

It is of vital importance to develop high caliber basic and clinical research programs which will place a premium on excellence both in patient care and education. Among other goals, basic pursuits will make it possible to culture the virus of hepatitis and develop an effective vaccine and chemotherapy which will then eliminate the growing problem of hepatitis; to develop artificial assists and identify mechanisms (besides nutritional deficiencies) which cause aregenerative phases of liver injury, and thereby increase longevity in irreversible liver disease; and to develop ways to prevent alcohol- or drug-induced liver injury by identifying mechanisms which increase susceptibility to liver injury in certain persons. It is expected that those working in the Stratton Laboratory in the future will, in the tradition and pace set by its current leader, continue to contribute greatly to such fundamental research.

This laboratory, however, must also provide the climate for much needed controlled therapeutic studies now greatly facilitated by computer analysis. Such investigations will have a significant effect on morbidity and mortality by objectively deciding the efficacy of available and anticipated medical and surgical regimens designed to treat the diseased liver and associated complications. Simultaneous epidemiologic, social, and psychological research on patients and their environment is necessary to provide a new order and reduce the incidence and complications of liver disease.

In concluding my comments on the Social Implications of Liver Disease, it is important to recognize that some will argue that special attention is not needed for liver disease, that cited implications may be used as an argument to increase support for many disease states which are of equal social importance, and that it is not possible to directly relate control of causative factors and reduction in mortality from liver disease. Against these arguments is the rapidly increasing mortality from completely preventable liver disease in the alcoholic, the demonstrated efficacy of ghetto-based special programs in decreasing environment-imposed liver injury, and our new-found moral and ethical responsibility to reduce the incidence of all illness in all people.

A major emphasis of The Mount Sinai School of Medicine, which has served as an illuminating guide to others, is the promise of equal attention to the biological and social aspects of medicine in your curriculum and practice. Social problems must be considered in our efforts to control most diseases, but especially liver disease. Recognition accorded by the Strattons to the desirability of aiding research underway in this institution on the liver and its diseases occurs at a most opportune time to help reorient priorities and achieve a healthier and happier society. The poet has aptly expressed this position when he said, "There is a tide in the affairs of men which taken at the flood leads on to fortune; . . . on such a full sea are we now afloat and must take the current when it serves, or lose our venture."

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Paroxysmal Supraventricular Tachycardia Produced by Routine Transvenous Pacing of the Right Ventricle

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Introduction

Supraventricular tachycardia has been induced experimentally by either applying atrial extra stimuli during atrial pacing, or ventricular extra stimuli during ventricular pacing (1–3). In these experimental studies, the stimuli had to be applied at specific intervals after the preceding paced beat. But, to our knowledge, the following is the first reported example of supraventricular tachycardia produced by routine external transvenous pacing of the right ventricle, and without regard to the timing of the stimulus.

Case Report

A 47-year-old white man was transferred to The Mount Sinai Hospital because of refractory tachycardia.

Seven years before he had begun to experience episodes of tachycardia, lasting two hours to three days. The episodes occurred approximately once every one or two weeks, but he tolerated them and was able to continue heavy physical work. Usually, he could terminate them with a Valsalva maneuver. He was given oral quinidine, 300 mg four times a day, and digoxin 0.25 mg per day, but the episodes continued.

Four months prior to admission he had an unusually prolonged paroxysm, accompanied by precordial discomfort. He was hospitalized, and the attack was terminated. He was subsequently given 30 mg propranolol per day, but after having two or three episodes of syncope, the dosage was decreased to 10 mg per day.

Three days prior to admission, he again had a prolonged episode of tachycardia and again was hospitalized. When his tachycardia could not be terminated with intravenous metaraminol, diphenylhydantoin, or procainamide, and responded only transiently to electric countershock, he was transferred to The Mount Sinai Hospital.

There was no history of alcoholism. A sister also experienced attacks of tachycardia.

Physical examination showed that the patient was well nourished and well developed. Blood pressure was 96/64, pulse 140 per minute and regular, and respirations 12 per minute and regular. The thyroid was not enlarged. There

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was no cervical venous distention, and the lungs were clear. The point of maximum impulse was in the 6th intercostal space near the anterior axillary line. The heart sounds were distant. A Grade II/IV short systolic murmur of the ejection type was present at the apex. The first sound was of normal intensity. The second component of S_2 was somewhat accentuated. No diastolic murmurs were present. There was no peripheral edema.

Laboratory Data: Hemoglobin (Hb) 14.7, Hct 43, WBC 7,400 with normal differential, ESR 3, BUN 24, glucose 130, Na, K, CL, CO_2 normal, bilirubin (total) 3.6, SGOT 130, LDH 235 (normal to 200), alkaline phosphatase 250 (normal to 85), PBI normal. Bilirubin, alkaline phosphatase, SGOT, and LDH subsequently returned to normal.

The film of the chest showed prominent pulmonary arteries and an enlarged left ventricle, and possibly an enlarged left atrium. The electrocardiogram (ECG) test administered on admission showed a supraventricular tachycardia at 125 per minute. The axis was vertical. There was clockwise rotation and nonspecific S-T and T wave changes in leads 2, 3 AVF and V_6. A subsequent ECG during sinus rhythm showed wide, notched P waves, suggesting right and left atrial enlargement and a P-R interval of 0.20. A diagnosis of primary myocardial disease was made.

Hospital Course: After admission sinus rhythm was restored with a precordial countershock of 100 watt-seconds' current, but the rhythm reverted to supraventricular tachycardia within a few minutes (Fig. 1A). He was started on digoxin, 0.25 mg per day orally, plus procainamide, 500 mg, then 1,000 mg, orally every six hours. The attacks continued, however, and the medication was changed to propranolol, 10 mg orally four times a day. A few days later quinidine was added in a dosage of 400 mg orally every four hours, and the attacks became less frequent and easily terminated with a Valsalva maneuver. After three days without attacks, he was discharged on an oral regimen of digoxin, 0.25 mg per day, quinidine, 400 mg every four hours while awake, plus a long-acting quinidine preparation at bedtime, and propranolol, 10 mg four times a day.

Four weeks after discharge, the patient again had a prolonged episode of tachycardia, with fatigue, lightheadedness, and near syncope, and was readmitted. The ECG showed supraventricular tachycardia at 140 per minute. A review of his medical history revealed that the patient had had lightheaded episodes prior to his taking propranolol, and therefore it was decided to administer this drug in increasing dosages. Because of the patient's history of syncope, however, and because his pulse rate during propranolol therapy fell below 60 per minute between attacks, a transvenous pacemaker electrode was passed to the right ventricle and attached to an Electrodyne Model TR-3 fixed rate external pacemaker.

During episodes of tachycardia, attempts to override his rhythm with the external pacemaker were unsuccessful. If, however, the pacemaker was turned on when he was in normal sinus rhythm, the following events were observed: The pacemaker stimulus was followed by ventricular capture, which was imme-

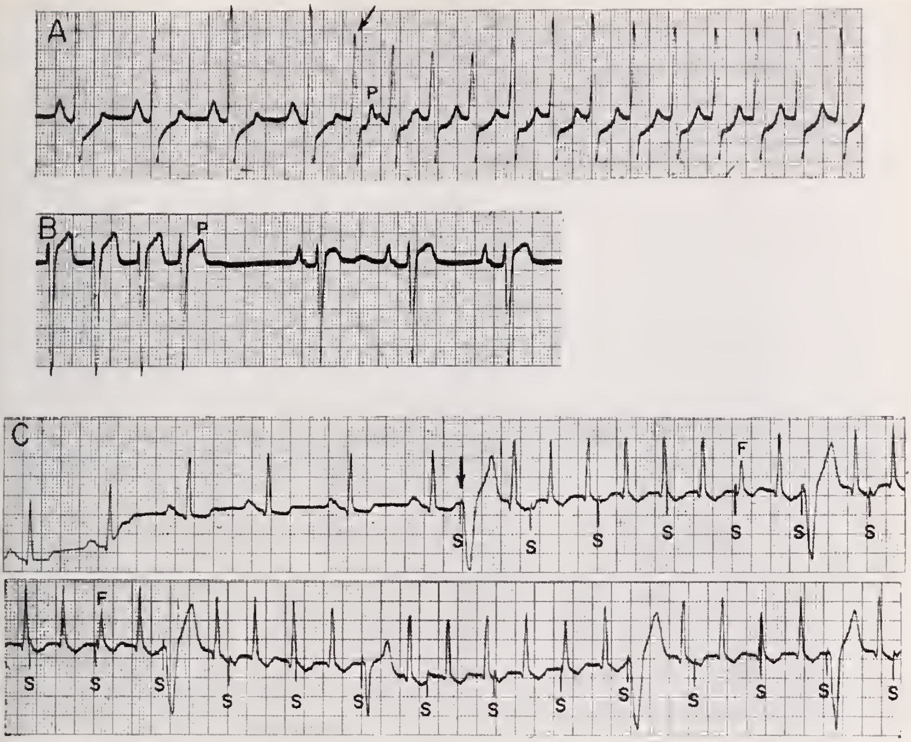

FIG. 1. A. Onset of spontaneous tachycardia (monitor lead). Arrow indicates first ectopic beat. A sinus P wave (P) is present after this first ectopic beat, suggesting its origin in the A-V junctional tissue. B. Spontaneous termination of tachycardia (lead V_2). Tachycardia ends with a retrograde P wave (P), also suggesting origin in the A-V junctional tissue. C. Pacemaker induction of the tachycardia (lead 2, continuous tracing). Arrow marks first pacemaker stimulus (S). After initiation of the tachycardia, the electronic pacemaker continues as a parasystolic focus with two fusion beats (F). Pacemaker rate 86/min.

diately followed by the supraventricular tachycardia (Fig. 1C). Esophageal leads at such times (Fig. 2A) showed that the ventricular captures (V) were followed by retrograde P (P) waves, at an R-P interval of 0.30 seconds. Valsalva maneuvers at this time were unsuccessful, but easily terminated the tachycardia as soon as the pacemaker was turned off (Fig. 2B). This series of events was reproduced on several occasions, and no attempt to produce the tachycardia in this manner was unsuccessful.

With a dosage of 50 mg propranolol every four hours, the patient had only rare, short episodes of tachycardia, which were easily terminated with a Valsalva maneuver. After three weeks with this dosage, and no episodes of lightheadedness or syncope, and no evidence of congestive heart failure, the pacemaker wire was removed. The patient was discharged with instructions to take 0.25 mg digoxin per day and 50 mg of propranolol every four hours.

On follow-up, four months after discharge, the patient was feeling well,

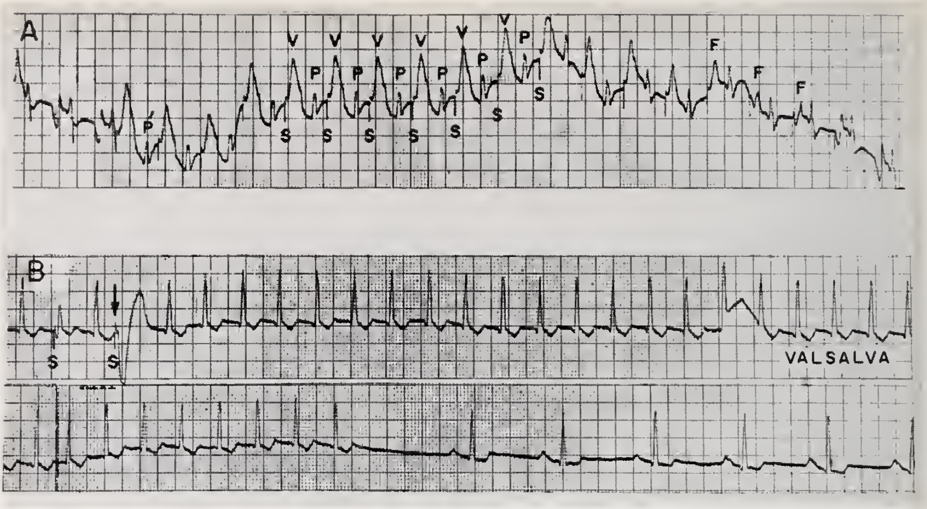

Fig. 2. A. Esophageal lead during pacemaker-induced tachycardia demonstrating intermittent pacemaker capture, with retrograde P waves. Ventricular capture is present after the third pacemaker stimulus, followed by an early P wave (P′), suggesting retrograde conduction of the ventricular capture to the atria. P-P′ interval 0.40 sec.; prior P-P interval 0.48 sec. Interval from beginning of pacemaker capture to retrograde P wave is 0.30 sec. F represents fusion beats. Pacemaker rate 120/min. B. Termination of induced tachycardia by a Valsalva maneuver after the pacemaker has been turned off (lead V_2). Arrow marks last pacemaker stimulus.

without symptoms or light-headedness. He was taking 80 mg propranolol four or five times a day, plus 0.25 mg digoxin per day. He had only occasional short episodes of tachycardia, which he could usually terminate easily.

Discussion

Since the supraventricular tachycardia consistently followed the first pacemaker stimulus, the tachycardia was probably induced by stimulation of the ventricles. This conclusion is also supported by the demonstration of retrograde conduction of the ventricular captures to the atria, when the P wave after the first ventricular capture was noted to appear early (P′ in Fig. 2A; see legend for details). Furthermore, the patient could not terminate his induced tachycardia with a Valsalva maneuver, as long as the artificial pacemaker was firing, but could easily terminate the tachycardia in this manner when the pacemaker was turned off (Fig. 2C). This, too, was demonstrated to be a consistent occurrence.

Supraventricular tachycardia has been induced experimentally by a number of investigators. Moe et al (3) were able to induce a paroxysmal atrioventricular (A-V) nodal tachycardia in a dog by applying atrial extra stimuli during atrial pacing. In their study, the tachycardia could be induced only if the extra stimuli were applied at specific intervals after the regular paced beat. The ventricular response to the atrial extra stimulus was delayed, and often aberrant. The tachycardia could also be terminated by appropriately timed atrial extra stimuli. From these observations, they concluded that the mechanism of production of the tachycardia was one of re-entry of an impulse through an A-V node capable of dual transmission, producing a self-sustaining tachycardia.

Durrer et al (2) were able to induce and terminate supraventricular tachycardia in a patient with complexes resembling those of the Wolff-Parkinson-White syndrome, by atrial extra stimuli during atrial pacing, and by ventricular extra stimuli during ventricular pacing. Again, based on the delay in appearance of the ventricular response to the atrial extra stimulus, and on the effect of this extra stimulus on the tachycardia, it was concluded that the mechanism was either a circus movement involving the Kent bundle, or a re-entry tachycardia initiated by "echo" beats from the A-V node.

Goldreyer and Bigger (1) were also able to induce supraventricular tachycardia in a patient by appropriately timed atrial extra stimuli. From their studies, they concluded that atrial re-entry was the most likely mechanism.

Our case differed from the above experimental studies in that this tachycardia was produced by routine external transvenous pacing of the right ventricle. Furthermore, in this case, stimuli occurring at various intervals after the preceding beat induced the tachycardia, whereas in the experimental studies the stimuli had to fall at specific intervals after the preceding beat.

A re-entry mechanism is favored when there is a prolongation of conduction, since it is postulated that a sufficient delay in transmission of an impulse through a portion of the atrioventricular node may allow adjacent nodal tissue to become repolarized and conduct the impulse back to the chamber of origin, producing an "echo" or "reciprocal" beat. Both atrial and ventricular reciprocal beats have been described, and ventricular reciprocal beats from electrical pacemaker stimulation of the right ventricle have also been reported (4, 5).

In this patient, retrograde conduction of the ventricular pacemaker captures to the atria was present (Fig. 2A), and the conduction time to the atria was prolonged to 0.30 seconds. This would favor a re-entry mechanism initiating the tachycardia, as was postulated in the experimental studies. The tachycardia could also have been produced by retrograde impulses reaching the atria in their vulnerable period. However, the tachycardia in this case was produced by stimuli which would reach the atria outside their vulnerable period. Furthermore, arrhythmias following such stimulation are generally irregular and terminate spontaneously after a few beats (6), whereas ours did not.

Summary

In a patient with paroxysmal supraventricular tachycardia, external transvenous pacemaker stimulation of the right ventricle during sinus rhythm regularly produced a supraventricular tachycardia. Retrograde conduction to the atria of the pacemaker ventricular captures was demonstrated, and the conduction time was delayed, suggesting re-entry as the mechanism of production of the tachycardia. Experimentally, supraventricular tachycardia has been induced by applying atrial and ventricular stimuli at specific intervals after the

preceding beat, but in this case the tachycardia was produced regardless of where the pacemaker stimulus fell in the cardiac cycle, and was produced during routine external transvenous pacing.

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Carcinoma of the Ileum 25 Years after Bypass for Regional Enteritis: A Case Report

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Evidence for the association of carcinoma of the small bowel with regional enteritis is gradually being accumulated; since 1956, eighteen instances have been recorded (1, 2). We now report another patient with adenocarcinoma of the ileum (with lymph node metastases) in regional ileitis of many years' duration. The important clinical point of this case is that the cancer occurred in the loop of ileum which had been bypassed 25 years before. We wish to call attention to the difficulties of diagnosis in this situation and to the potential danger of long-standing intestinal bypass.

Case Report

A 55-year-old woman with 4 months of nausea, vomiting, and diarrhea was admitted to The Mount Sinai Hospital on November 27, 1969. Twenty-five years earlier she had experienced lower abdominal pains and low-grade fever. X-ray examination of the gastrointestinal tract was said to be normal and chronic appendicitis was the diagnosis. Six months later (July 1944), severe right lower quadrant pain with fever developed. At laparotomy, regional ileitis (Crohn's disease) was recognized. No tissue was resected but drainage persisted for several weeks. In July 1945, following severe abdominal pains, side-to-side ileotransverse colostomy in continuity was done. Because of obstruction, this was revised in the postoperative period to a side-to-side ileodescending colostomy. The proximal colon and excluded bypassed terminal ileum were left in place.

For the next 24 years she did well, having one to seven bowel movements per day, maintaining stable weight, and enjoying a full, active life. She married but had no pregnancies.

In July 1969, abdominal cramps with increasing stool frequency led to x-ray examination of the gastrointestinal tract. No obstruction to the flow of barium was seen. However several gas-filled dilated loops were noted and felt to be blind loops. The excluded bowel was not well seen. Despite treatment with antibiotics and Azulfadine®, the symptoms persisted. Nausea and vomiting started and severely prolapsed hemorrhoids developed. Because of dehydration, she was admitted to a local hospital and then transferred to The Mount Sinai Hospital.

On physical examination, her pulse was 84, blood pressure 118/72, respiration 20, temperature 98.6°F, weight 95 pounds. She was thin, non-icteric, and in no acute distress. The abdomen was soft; no masses or viscera were palpated. Normal bowel sounds were heard. There was slight tenderness in the right lower

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quadrant. The initial impression was partial obstruction with dilated blind loops and reactivation of disease in the bypassed segments.

Laboratory Data: Hemoglobin (Hb), 12.5; WBC, 8,200; segmented, 52; bands, 11; lymphocytes, 34; monocytes, 2; platelets, 418,000; ESR, 25; prothrombin time, 14.9/12; urine: specific gravity 1006; protein-trace; glucose, negative; WBC 1–2; Serum Ca 9.3; P, 3.5; glucose, 100; BUN, 9; uric acid, 4.5; cholesterol, 200; bilirubin, 0.3; alkaline phosphatase, 40; LDH, 140; SGOT, 30; Vitamin A, 37 mg %; carotene, 46 mg %; D-Xylose excretion in 5 hours, 5.3 gm; total protein, 5.4; albumin, 2.4; IVP, normal. The obstructive series revealed some dilated loops of small bowel with air fluid levels, suggestive of incomplete small bowel obstruction. ECG was normal.

Because of her symptoms and the preoperative diagnosis of intestinal obstruction due to disease in the excluded segment, the patient underwent exploratory examination. At laparotomy the excluded terminal ileum and cecum were resected, as well as the dilated blind loop of the ileodescending colostomy. The site of the original ileotransverse colostomy was closed. The excluded terminal ileum and its mesentery were erythematous and markedly indurated and when the ileum was opened, active transmural inflammatory disease with ulceration was discovered. Though no granulomata were identified, the histology of the resected gut was that of regional ileitis. In addition, an adenocarcinoma with extension to the serosal surface and metastases in two lymph nodes was found in this segment.

In the postoperative period, the patient's appetite remained poor, she became edematous; diarrhea developed. A subphrenic abscess, which also developed, was drained. Despite antibiotics and supportive measures, she continued to decline and died on the 31st day after the initial surgery. There was no postmortem examination.

Comment

It is generally believed that the risk of carcinoma increases in patients with ulcerative colitis. Evidence regarding this association in regional enteritis is now emerging. Wyatt (2) has argued that the association of such a rare neoplasm as carcinoma of the small bowel with regional enteritis, and the presentation of the neoplasm at a younger age than other small bowel cancers, leaves little doubt that the neoplasm is somehow related to the inflammatory bowel lesion.

We wish to stress two points arising from consideration of the case. First, the presence of a clinically silent neoplasm in a bypassed or excluded loop of intestine is extremely difficult to diagnose radiologically since the lesion is out of the main (barium-filled) stream. Second, five of the previously recorded neoplasms occurred in bypassed, although not necessarily completely excluded, loops of small bowel. Although cumulative experience at this institution has shown that the bypass usually leads to quiescence of the inflammatory process in granulomatous enteritis, the cases cited, as well as our own, emphasize the possible long-term dangers inherent in this type of surgery.

In this context, it is of interest that Marshak and Lindner, in their monograph on the radiology of the small bowel, illustrate an adenocarcinoma of the jejunum occurring in a surgically constructed blind loop of seventeen years' duration (3).

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Brachial Plexus Compression by Excessive Callus Formation Secondary to a Fractured Clavicle: A Case Report

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The earliest structure to be recognized as a cause of brachial plexus compression was the cervical rib, which Adson stated was known to Galen and Vesalius (1).

In 1938 Naffziger popularized and coined the term "the scalene syndrome" (5) to describe the condition of brachial plexus compression secondary to hy-

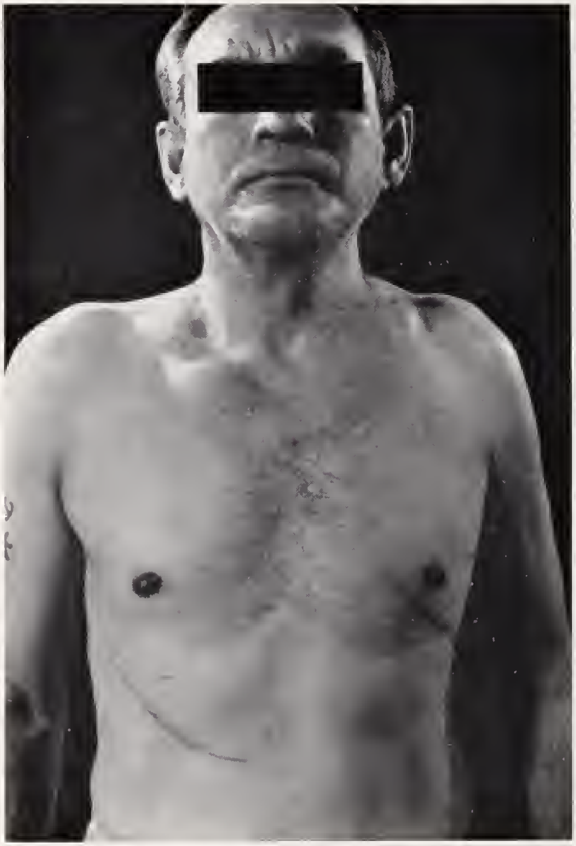

Fig. 1

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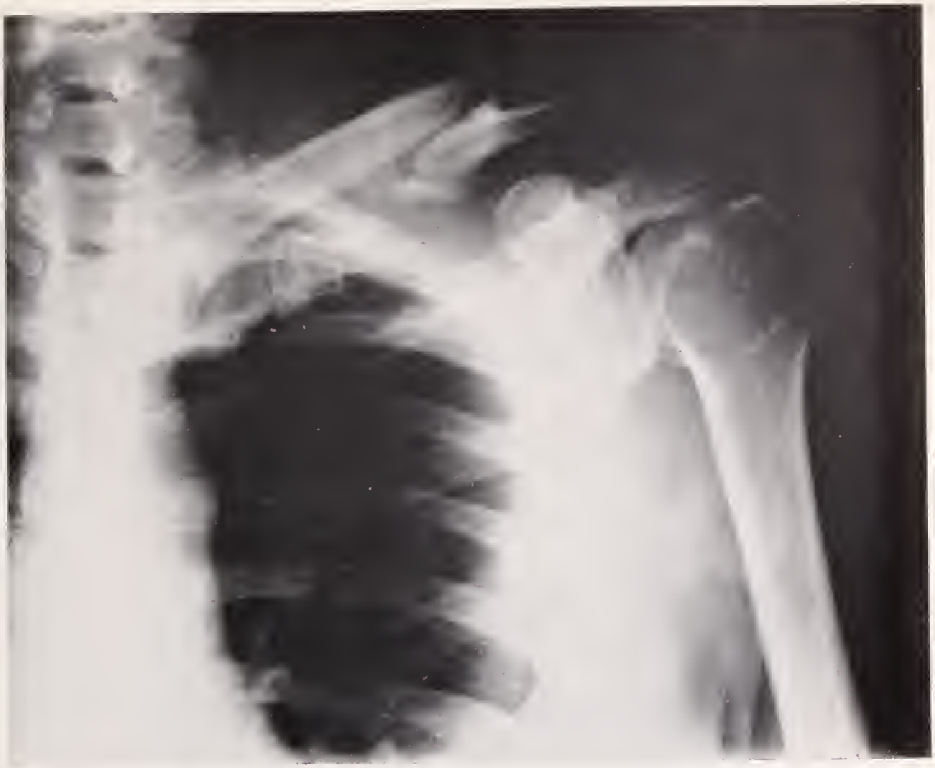

Fig. 2

pertrophy of the anterior scalene muscles; since then, other structures in the thoracic outlet have been incriminated.

The following is a case report of brachial plexus compression resulting from excessive callus formation secondary to a fractured clavicle. Although brachial plexus injuries have been demonstrated in pseudoarthrosis of the clavicle as a result of the deformity, rarely has a large callus formation been incriminated in such lesions.

Case Report

V.G. is a 46-year-old man who fell on October 6, 1968 and fractured his left clavicle. He was treated with a figure-eight plaster cast which was removed December 2, 1968. At the time of injury and upon removal of the cast, the patient was reportedly without neurological deficiency. Shortly after removal of the cast, however, he began to experience sharp pains and paresthesias along the medial aspect of his left arm and all the fingers of his left hand. In addition, he noted progressive weakness of the left extremity. His medical history included mild diabetes (well controlled on Tolbutamide®).

Examination revealed a firm, non-tender, non-movable mass in the left supraclavicular area, attached to the middle third of the clavicle (Fig. 1). There

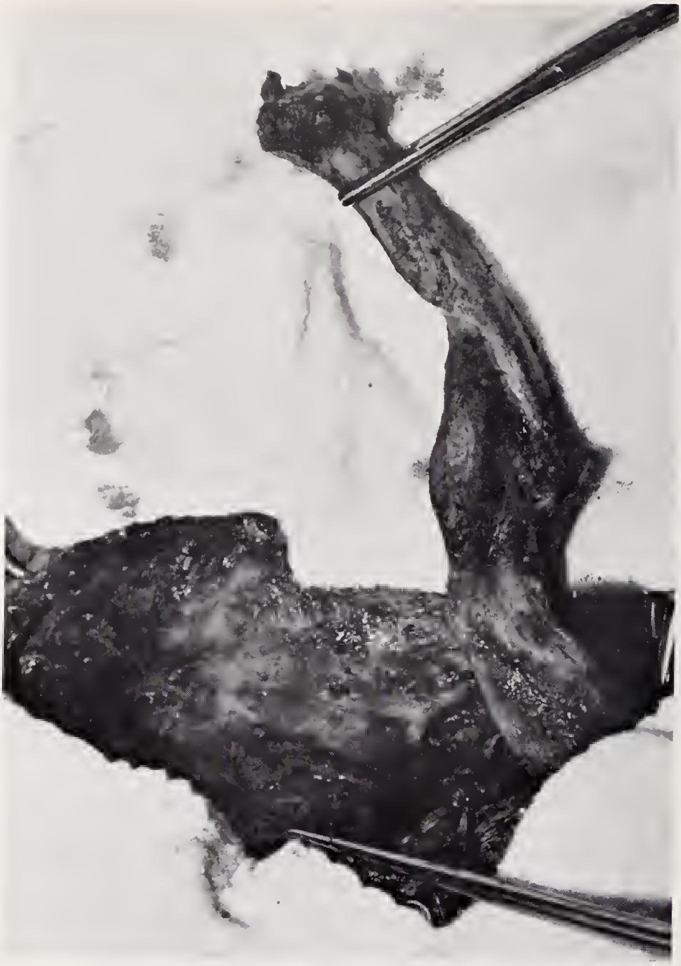

FIG. 3

was severe atrophy and weakness of the left deltoid, biceps, triceps, flexors, and extensors of the wrist and intrinsic muscles of the hand. The serratus anterior muscle was normal. There was hypasthesia to pin prick over the entire left arm. The Adson test and forced depression tests were positive on the left.

Plain x-ray films and tomograms (Fig. 2) of the left clavicle showed a large callus formation. Electromyography examination revealed denervation potentials of the muscles supplied by C5, 6, and 7.

The clavicle was removed entirely at surgery (Fig. 3). Immediately after the operation, all pain and paresthesias described in the left upper extremity disappeared. Two weeks after surgery, the patient noted considerable motor improvement. Seven weeks after the operation, another electromyography examination showed marked improvement. The patient reported no functional impairment following removal of the clavicle.

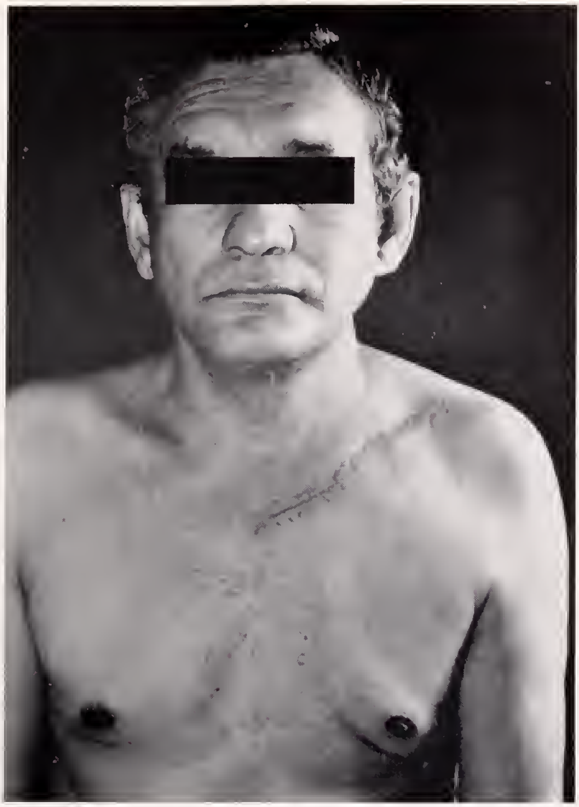

FIG. 4

Discussion

This case illustrates one of the more unusual causes of brachial plexus compression. Its pathogenesis can best be understood if one fully understands the anatomy of the shoulder girdle. In the normal individual, the sternoclavicular angle ranges between 15 and 30 degrees. An angle of this dimension allows free egress of the neurovascular bundle anterior to the first rib and posterior to the arched middle third of the clavicle. When the shoulder is allowed to droop, the outer end of the clavicle descends and the sternoclavicular angle decreases, narrowing the costoclavicular space and pinching the neurovascular bundle between the clavicle and the first rib. This phenomenon can be demonstrated by palpating the radial pulse while drawing the upper extremity down and backward. This test is considered positive upon obliteration of the pulse and reproduction of the symptoms. However, this maneuver may be positive in a number of "normal" individuals and thus cannot be considered pathognomonic.

In this case, it is easy to see why the symptoms developed in the patient only after removal of the cast. While in the cast, his shoulders were kept elevated. However, when the cast was removed, his shoulders drooped. Since the callus was unusually large, the symptoms developed rapidly.

Total removal of the clavicle was the chosen procedure for decompressing the brachial plexus because the clavicle may be readily spared without functional impairment and little or no cosmetic deformity (Fig. 4).

Summary

An unusual case of brachial plexus compression secondary to excess clavicular callus, treated by total removal of the clavicle, has been presented.

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Behcet's Syndrome: A Case Report

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Introduction

In 1937 a Turkish dermatologist, Behcet (1), described a syndrome presenting a triad of recurrent oral, genital, and ocular lesions. Reference to the entity goes back to antiquity, indeed to Hippocrates in his Third Book of Endemic Diseases (2). In 1930 Adamantiades (3) had described a case which fulfilled the criteria which Behcet later established.

A patient with this syndrome was recently studied at the Gynecologic Service of The Mount Sinai Hospital. In view of the rarity of this disease, this case report and brief review of the literature is presented with the hope that awareness of this entity and its potentially serious neurologic manifestations will enable others to recognize this disease and begin prompt therapy.

Case Report

M.A. was a 36-year-old Puerto Rican woman, para 4004, who was first seen in February 1968 because of an asymptomatic suburethral lesion. The physical examination, except for the presence of a friable 2 cm necrotic papillary lesion of the anterior vaginal wall ½ cm from the introitus and from an intrauterine coil in place for two years, was within normal limits. A Papanicolaou smear, serologic test for syphilis, and complement fixation test for lymphogranuloma venereum were all negative. A biopsy revealed an ulcerated granulomatous lesion, without Donovan bodies. Aerobic and anaerobic cultures of the urine and of the vaginal lesions were negative, and urinalysis, blood count, blood chemistries, and chest x-ray were within normal limits. The patient was treated with tetracycline for ten days without effect.

On April 11, 1968 she was readmitted to the hospital. At that time she had a 1 cm healed ulcer on the left buttock, and a 1.5 cm necrotic gray ulcer on the left anterior vaginal wall near the urethra. Intravenous pyelography and cystoscopy revealed only mild trigonitis. Biopsies of the vaginal lesion again showed a nonspecific granulomatous process. One pathologist thought them to be compatible with leishmaniasis. Further biopsies reviewed here and at the Armed Forces Institute of Pathology were nondiagnostic. Titers for lymphogranuloma venereum, tularensis, and brucellosis were negative, as were multiple cultures. On May 2, 1968, a cholecystectomy was performed for chronic cholecystitis and cholelithiasis. The patient recovered uneventfully from this procedure, but the necrotic vaginal ulcers increased in size.

During the next two months, she was treated with sitz baths, nalidixic acid, erythromycin, ampicillin, and sulfasuxasol in full courses. Tetracycline was

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given for 30 days. She also received a course of treatment in the Hyperbaric Chamber. The ulcers increased in size and number; extreme pain upon urination necessitated the insertion of a Foley catheter. On June 22 a second strength PPD was positive, but all cultures, including those for acid fast bacilli, were reported negative. On June 29 the patient developed exudative tonsillitis that responded to penicillin. On July 8, 1968 ulcers of the vagina and left labium were excised with the hope of alleviating her symptoms. She was now unable to walk or move her legs because of pain. For a few days she had some relief, but within five days of surgery the incised area started to necrose.

On July 17, 1968 herpetic ulcers of the mouth appeared for the first time. These were treated with palliative mouth washes and analgesics. On July 18 pelvic examination revealed a shaggy ulceration which involved extensively the lower two-thirds of the inner aspect of the left labium major and the lower one-third of the vagina spilling onto the fourchette. The entire left vulva was firm with tender induration. Oral ampicillin and local hygiene were started but this regimen was also unsuccessful. At about this time it was suggested that this might be a case of Behcet's syndrome. Since all medications had failed to control these lesions, on July 27, 1968 the patient was started on a course of prednisone 20 mg three times a day. After ten days this was decreased to 10 mg three times a day, and two days later to 5 mg three times a day. Tapering was continued so that by August 15 she was given 5 mg daily. By July 31, 1968, just four days after commencement of treatment, there was subjective improvement. By August 4 there was objective improvement. On August 24, 1968 the patient was asymptomatic with completely healed ulcers, and was discharged on a maintenance dosage of 5 mg of prednisone daily.

She was treated as an outpatient and after 4½ months the steroids were discontinued. Six weeks later she had a recurrence of the oral and genital ulcers which was successfully treated with steroids. She continues on steroid therapy as an outpatient to this date.

Discussion

Behcet's syndrome which has been reported in the modern literature is a triple symptom complex of oral and genital ulcers and hypopyon of the eye (4–11). The diagnosis is made by the appearance of any two of these three major manifestations. The skin, nervous system, pericardium, veins, and joints, may also be involved.

The mouth and oropharynx (4, 6–8, 10) are involved in 90 percent of the cases reported. Multiple ulcers of varying size with punched-out edges and a grey necrotic base are seen. They may be surrounded by a halo of erythema but the mucous membrane surrounding the lesion is otherwise normal. Pain and dysphagia are common.

The genitalia (4, 6, 7, 9, 10) are involved in 84 percent of the cases reported (21 of 25 females) and show similar ulcerations. In the male the scrotum, or less often the shaft of the penis, or glans, is involved. Epididymitis may also occur. In the female the labia, and less often the vagina, or cervix, are involved.

Ocular manifestations occur in 80 percent of the cases. Hypopyon iritis is the most common ocular lesion. Chorioretinitis and exudative retinal detachment may occur late (7, 12). There may also be a periarteritis and endarteritis of the retinal vessels, iridocyclitis or optic nerve atrophy (10–13).

In 1941 Jensen (7) noted that persons with Behcet's syndrome had a peculiar skin sensitivity. They demonstrated a positive reaction to an intradermal injection of saline. This peculiar skin sensitivity had previously been noted by Blobner (14) and has since been confirmed by others (7, 11, 15). Erythema nodosa, folliculitis, pustules, furuncles, and pyodermic abscesses may also appear (4, 7, 10, 11, 16).

Pericarditis has been associated with this syndrome (10, 16, 17). Venous thrombosis, particularly of the lower extremities, has been reported also (7, 10, 16, 18).

Dowling (7) found central nervous system involvement in 19 percent of his patients. Such involvement increases greatly the mortality and morbidity (7, 10, 11, 17, 19), with 47 percent mortality recorded by Schotland (19). With the exception of the lower motor neuron, any part of the neuroaxis may be involved (18).

The etiology of this syndrome remains unresolved. France (6) believed a vasculitis to be the underlying pathology. On skin biopsy, he showed venous thromboses and inflammation within the vascular walls. Because of this and the finding of thrombophlebitis in 9 of 33 cases, he theorized a vascular basis for this disease.

Others (20) have been able to produce arthritis, skin and labial ulcers, and iridocyclitis in rats by the intracutaneous injection of Freund's adjuvant. Because of this and the presence of erythema nodosum in many patients, an allergic etiology has been proposed.

The most commonly accepted etiology is a viral one (4, 10, 21). Sezer (21) was able to produce encephalitis in mice by intracerebral, peritoneal, or subcutaneous injection; and he produced chorioretinitis in rabbits by the intraocular injection of a viral culture obtained by growing material taken from subretinal exudate and corpus vitreum of infected patients on the chorioallantoic membrane of a developing chick embryo. He was unable to produce experimental disease by using material taken from the anterior chamber of the eye or from aphthous ulcers. The virus, which readily traverses a double-padded Sietz filter, grows well on the chorioallantoic membrane of a developing chick embryo. Similar lesions were produced by inoculation with material obtained by grinding in saline the blood clot from a person with an exacerbation; this is thought to indicate the presence of a viremia. Furthermore, he was able to pass this lesion serially. Sezer (21) and others (22) have demonstrated the presence of virus-neutralizing antibody. Evans et al (22) could grow this virus on the chorioallantoic membrane of a developing chick embryo but were unable to transfer the virus to human embryo kidney cells, human amnion cells, or Hela cells. They were also unable to transmit the agent to mice, guinea pigs, or rabbits by intracerebral, intramuscular, or intraperitoneal injections. Thus, while there is much evidence of a viral etiology, it is by no means conclusive.

Summary

1. A case of Behcet's syndrome is presented with oral and genital ulcers successfully treated with corticosteroids.

2. The major manifestations of this syndrome are oral and genital ulcers and hypopyon of the eye. The skin, vascular system, neurologic system, venous system, and pericardium may also be involved.

3. While the etiology remains obscure, there is some evidence that this may be a viral disease.

4. The only known treatment is corticosteroids.

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Myasthenia Gravis and Focal Glomerulonephritis Following Idiopathic Thrombocytopenia Purpura: A Possible Common Etiology?

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ARTHUR SAWITSKY, M.D.†

Introduction

An autoimmune origin of idiopathic thrombocytopenia purpura (ITP) is very likely. The demonstration of a plasma antiplatelet factor with characteristics of an antibody, and the relation with lymphoproliferative disorders and systemic lupus erythematosus, as well as the response to corticosteroids, are evidence for an immunologic pathogenesis (1).

The hypothesis has been advanced that myasthenia gravis is an autoimmune disease (2, 3) in which presumably abnormal lymphoid clones may directly, or through antibodies, attack the A and I bands of striated muscle and myoid cells of the thymus (4). As yet, the motor end-plate has not been implicated. The increased incidence of diseases with immunologic disturbance such as lupus erythematosus, rheumatoid arthritis, and thyroiditis in myasthenia patients has also been pointed out (4). It has also been recently pointed out that soluble immune complexes depositing on glomerular basement membranes are important in the pathogenesis of glomerulonephritis (5).

The development of myasthenia gravis, a disease of presumed altered immunity and often associated with thymic abnormalities, followed by focal glomerulonephritis, a disease of possible immunologic pathogenesis, in a patient with previous idiopathic thrombocytopenia purpura, another disease of presumed altered immunity, forms the basis of this report.

Case Report

A thirty-year-old white man was first seen in June 1960, because of the spontaneous onset of epistaxis and petechiae. There was no history of drug ingestion, exposure to toxins, or recent infection. The patient had previously been in good health and had undergone an uneventful appendectomy at age seven. Family history was noncontributory.

Physical examination at that time was within normal limits except for generalized multiple petechiae. Laboratory data revealed a hemoglobin of 15.6 gm%, hematocrit of 46%, white blood cell count of 9,300/cu mm with a differential

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white blood cell count of 66% polymorphonuclear cells, 23% lymphocytes, 10% monocytes, and 1% eosinophils. The platelet count was 60,000/cu mm. Urinalysis, fasting blood sugar, blood urea nitrogen, uric acid, prothrombin time and VDRL were within normal limits. A serum electrophoresis showed a mild increase in alpha$_2$ and beta globulins. The Coombs' test and lupus preparations were negative.

The patient refused a bone marrow examination and a tentative diagnosis of idiopathic thrombocytopenic purpura was made. He was then started on prednisone, 60 mg daily, with little improvement in the platelet count over a six-month period and in January 1961 a splenectomy was performed. The platelet count rose briskly following splenectomy. On the seventh postoperative day the patient suffered a pulmonary embolism from which he made an uneventful recovery. At that time the platelet count was 1 million/cu mm. The platelet count became normal and has remained so. During this entire period the hemoglobin and white blood cell count have remained normal.

In September 1967, the patient started experiencing diplopia and weakness after activity. The edrophonium (Tensilon®) test was positive and myasthenia gravis was diagnosed at the Myasthenia Gravis Clinic of The Mount Sinai Hospital, New York City. The patient's disease was controlled with pyridostigmine bromide (Mestinon®). Muscle, thymic, and thyroid antibodies were absent in the patient's serum.

In November 1968, he was admitted to another hospital for sudden onset of generalized anasarca. Urinalysis showed 2+ proteinuria with a specific gravity of 1.022 and rare RBC. A twenty-four hour urine collection revealed 2.5 gm% of protein. Blood urea nitrogen was 23 mg%, albumin was 2.8 gm% and globulin 3.2 gm%. Total cholesterol was 395 mg%. Chest x-ray and intravenous pyelogram were negative. Skin biopsy was nondiagnostic. Kidney biopsy revealed focal glomerulonephritis (Fig. 1). Immunofluorescent antinuclear antibody revealed a homogeneous 2+ positive pattern, but repeated lupus preparations and immunoglobulin levels have been normal. VDRL and Coombs' tests were negative and the PBI and liver function tests were normal. Lymphocyte transformation, chromosome analysis, and tritiated thymidine incorporation into the transformed lymphocytes were normal. The patient has continued having proteinuria and hypoalbuminemia, but was able to return to work. Repeated blood counts and platelet counts remained normal.

Discussion

Idiopathic thrombocytopenia purpura has been thought of as an autoimmune disorder since 1951, when Harrington and his associates demonstrated that plasma or blood from these patients produced severe thrombocytopenia when infused into normal recipients (1). The presence of an antiplatelet factor in the plasma of such cases was, and still is, the main point of evidence that this disorder is an autoimmune disease. Shulman has more recently demonstrated that this antiplatelet factor is present in the 7S gamma globulin fraction, that it

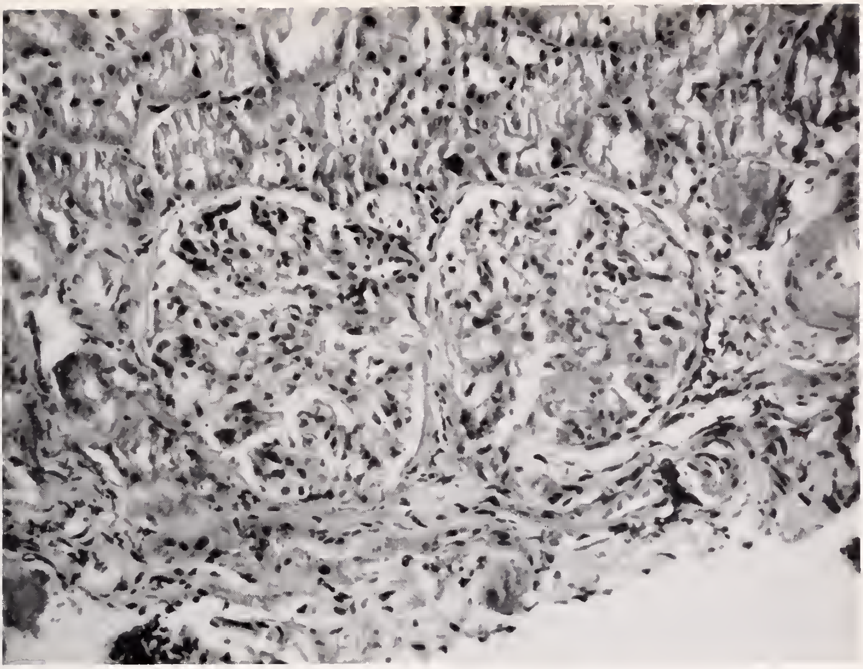

FIG. 1. Kidney biopsy showing focal glomerulonephritis. (Hematoxylin and Eosin × 200)

can be absorbed by normal human platelets, is species specific, and that it affects autologous as well as homologous platelets (6).

The association of ITP with lupus erythematosus and lymphoproliferative disorders lends further support to an immunologic pathogenesis (1). The increased incidence of ITP in thyrotoxicosis has led to the speculation that these diseases may have a similar immunologic background and that the etiologic agent in ITP is similar to LATS in thyrotoxicosis (7). Based on some of these findings, Baldini has recently proposed that the term immunologic thrombocytopenia replace the idiopathic thrombocytopenia (1).

There is increasing evidence that the thymus has a central role in the development of the immune system (8). Burnet has postulated that a disturbance of immunologic functions of the thymus leads to production of autoimmune disease (9). Pathologic thymic abnormalities are found in 75 percent of patients with myasthenia gravis (10), and sera of myasthenic patients contain a significantly increased incidence of antibodies including a 7S gamma globulin that binds to the lateral A and I bands of mammalian skeletal muscle and cross reacts with certain thymic epithelial cells (11). These so-called thymic epithelial cells have now been identified as thymic myoid cells (12). Myasthenia gravis is also associated with diseases having disturbed immunologic function such as lupus erythematosus, rheumatoid arthritis, thyroiditis, pernicious anemia (4), and chronic lymphatic leukemia (13).

The report of Coombs' positivity in certain of the thymoma patients with erythroid aplasia or pancytopenia has suggested that this state which is sometimes associated with myasthenia gravis may also be a consequence of autoimmunity (14).

Because of the pathologic thymic changes, the occurrence of autoantibodies, and the frequent association with disease thought to be autoimmune in nature, the suggestion has been made that autoimmune mechanisms play an important pathogenetic role in the development of myasthenia gravis.

The recent induction of glomerulonephritis in monkeys employing human anti-glomerular basement membrane antibody eluted from human nephritic kidneys appears to establish the pathogenicity of these antibodies in some cases of glomerulonephritis (15). Focal glomerulonephritis has been noted in some cases of lupus erythematosus where the glomerular lesion now appears to be due to deposits of soluble complexes of anti-DNA antibodies and DNA (16). However, focal glomerulonephritis is a nonspecific finding and is associated with many other clinical conditions (17). It is, therefore, difficult in this patient, in whom electron microscopic and immunologic studies were unfortunately not performed upon the kidney biopsy, to unequivocally state the exact nature of his renal disease.

Simpson has pointed out that both myasthenia gravis and lupus erythematosus may have a similar immunologic pattern (although each disease is clinically distinct) (4). Antinuclear antibodies, as present in the serum of our patient, were present in 20% of patients with myasthenia gravis in Simpson's series and 15% of myasthenics in Adner's series (18). Thus, it appears that antinuclear antibodies are frequently present in myasthenia gravis without other evidence of lupus erythematosus.

We also note that to our knowledge, the association of idiopathic thrombocytopenia purpura, myasthenia gravis, and focal glomerulonephritis have previously not been described either independently or as occurring in lupus erythematosus.

The development of myasthenia gravis and focal glomerulonephritis in a patient with previous idiopathic thrombocytopenia purpura raises the possibility that an underlying defect (perhaps thymic) permitted the development of "forbidden" clones of cells that produced autoantibodies initially affecting platelets and later muscle and glomerulus.

Summary

The development of myasthenia gravis and focal glomerulonephritis following idiopathic thrombocytopenic purpura, diseases of presumed altered immunity, is discussed as a common underlying thymic defect permitting the development of "forbidden" clones that produced autoantibodies in serum initially affecting platelets and later muscle and then glomerulus.

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Medical Therapy for the Cerebrovascular Insufficiencies: Eight Years' Experience

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For the past eight years cerebrovascular insufficiencies of diverse origins have been studied and treated. The group now totals 61 cases. The method of treatment consists of a combination of Dilantin®, carotid sinus therapy, and support stockings. These simple measures avoid the risks of surgical intervention, the hazards of anticoagulants, and, if proved effective, might be the method of choice.

We present seven illustrative cases of varied types drawn from the series now totalling 61, and a brief discussion.

Case Report

1) A 65-year-old man was admitted to The Mount Sinai Hospital because of a two- to three-month history of episodes of sudden difficulty in expressing himself. He also had many episodes of paresthesias of the right hand and wrist. One episode of syncope had occurred. Examination revealed normal blood pressure and no postural hypotension. Compression of the right common carotid artery produced paresthesias and clonic movements of the left upper extremity. Massage of the right carotid sinus produced contralateral paresthesias only. There was no change in pulse or blood pressure. While hospitalized, several episodes of dysphasia and dysarthria occurred, with residual speech defects. The paresthesias became a daily occurrence and began involving the left hand also. Right femoral angiography revealed complete occlusion of the left internal carotid artery and partial occlusion of the left external carotid artery (Fig. 1). He was treated with support stockings, Dilantin®, and atropine. No further episodes of speech impairment occurred. The paresthesias became much less frequent. Because of a history of benign prostatic hypertrophy and continuing symptoms, a transurethral resection was performed without difficulty. He has continued to do well and has been followed for one year.

2) A 59-year-old man had intermittent claudication for several years, a duodenal ulcer, a duodenal tumor, an elevated serum uric acid, and occasional episodes of dizziness. After one week of blurred, "wavy" vision bilaterally, dizziness, poor balance, and weakness, he was admitted to another hospital. While in the hospital dizziness, dysarthria, and a right hemiparesis developed. He was treated with anticoagulants, oral vasodilators, and carbon dioxide inhalation. Several minutes later, a left hemiparesis appeared. Nystagmus was noted while he was receiving barbiturates but this was subsequently noted when he was not receiving barbiturates. A stool guaiac test was 2 positive. Right brachial and

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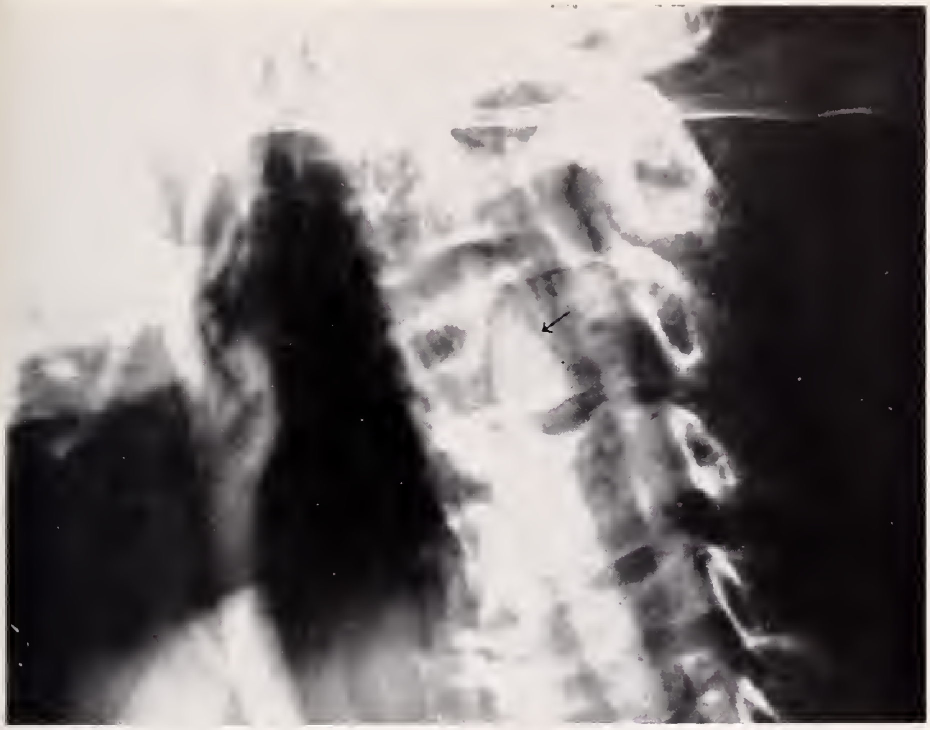

Fig. 1. Complete occlusion of left internal carotid artery (Patient #1).

left carotid angiography revealed occlusive disease of the basilar artery; both anterior cerebral arteries filled from the right internal carotid artery; the right posterior cerebral artery filled from the right internal carotid artery. Discharged to the care of his physician, the patient was still on anticoagulants, but purpura developed in the right forearm and inguinal areas. When the patient was seen by us in consultation, anticoagulants were stopped. Soon thereafter he was admitted to The Mount Sinai Hospital. Examination revealed a normal reclining blood pressure with a slight postural hypotension without symptoms. Compression of the right common carotid artery produced a "dim" effect on the vision of the right eye. Compression of the right carotid sinus produced dizziness, perspiration, bradycardia, and a decrease in blood pressure. He was treated with support stockings; hydrochlorothiazide was added subsequently when the blood pressure became elevated (170/100). Atropine and Dilantin® were added following an episode of dizziness at a time when bilateral carotid sinus reaction could be demonstrated. No further episodes of dysarthria or hemiparesis occurred. Visual symptoms are now infrequent and slight. Dizziness occurs rarely. The patient has been followed for six years.

3) A 74-year-old woman developed dizzy spells "about twice a day" in the two months prior to her first visit. She had also had bilateral tinnitus for years. One year ago she was said to have had a right-sided Bell's palsy. She also had

hypertensive cardiovascular disease with angina and one episode of paroxysmal atrial flutter. On examination there was rotary nystagmus on right lateral gaze. The right nasolabial fold was slightly shallower than the left. There was slight postural hypotension with dizziness. Compression of the right common carotid artery for one to two seconds only, produced severe dizziness. Compression of the left common carotid artery produced a similar but less marked response. No change occurred in pulse or blood pressure. Right and left brachial angiography was unsuccessful. Left axillary angiography showed a left vertebral artery that was described as being either occluded or hypoplastic. The patient refused further studies. Support stockings and atropine were begun (she was also receiving hydrochlorothiazide and reserpine). Her episodes of dizziness became very infrequent. She has been followed for three years.

4) In a 65-year-old man, a sudden left hemiparesis and left-sided paresthesias developed five weeks before his initial visit. Two weeks later, a similar episode involved the right side, along with shaking of the right hand. Two weeks later, another left-sided episode occurred. Three days later, another right-sided episode occurred, with impaired speech. He had a history of hypertension, had a severe dizzy spell 3 years ago, and in the past one to two years he had been awakened twice by a "shock-like feeling—like being jarred." One year ago, while working, he experienced chest pain. He also had benign prostatic hypertrophy. Examination revealed a bradycardia (44–56). Compression of the left common carotid artery produced "slightly cloudy" vision in the left eye. Compression of the right carotid sinus produced dizziness and numbness in the *right* hand and "slightly cloudy" vision in the right eye. No change occurred in either pulse or blood pressure. Compression of the left carotid sinus produced dizziness and numbness in the *left* hand and "slightly cloudy" vision in the left eye. No change occurred in pulse or blood pressure. An electrocardiogram revealed the presence of an old myocardial infarction. Left brachial angiography revealed occlusion or hypoplasia just below the region of the vertebrobasilar junction. Right brachial angiography revealed filling of the right vertebral artery and basilar artery, but no communication with the left vertebral artery. The right posterior cerebral artery filled from the right internal carotid artery. There was no definite link between the right posterior cerebral artery and the basilar artery. Support stockings, Dilantin®, and atropine were begun. He improved markedly. Later, prostatic symptoms gradually increased and one year later, a transurethral resection was performed. He again felt well until one year later, when a second myocardial infarction occurred. He recovered, continued on the above regimen, and has felt well since then. He has been followed for four years.

5) A 66-year-old man had four severe dizzy spells with falling, during the year prior to his first visit. One of these episodes was also accompanied by vomiting. In the past one to two years, he noted impaired hearing and tinnitus bilaterally. For the past two to three years, he noticed occasional tingling of both hands, arms and shoulders—just before voiding and also at the onset of voiding. On examination there was slight impairment of tandem walking ability. There was slight postural hypotension without symptoms. Compression of either

carotid sinus area for one to two seconds only, produced asystole and a "little sway." Massage of either carotid sinus produced similar results. Blood pressure readings were not taken. Left brachial angiography was normal. He was treated with support stockings and Dilantin®; his symptoms decreased but were still troublesome. Atropine was added and he felt somewhat better for nine months, then the dizzy spells began recurring. He therefore received radiotherapy to both carotid sinus areas. No more episodes have occurred since that time (nine months) and he has felt very well.

6) A 38-year-old man had two episodes of micturition syncope in four weeks. In the first episode he struck his head. The second episode was followed by chest pain. During this 4-week period he also had occasional dizziness while walking. The history revealed one previous episode of micturition syncope at approximately age ten. One year ago he had had a grand mal convulsion and was hospitalized elsewhere; no cause for the convulsion was found. On examination, a bradycardia of 56 was noted. He was not an athlete nor was he taking medication. There was a slight postural hypotension with dizziness. Bending and straightening also produced dizziness. Compression of either common carotid artery produced dizziness and a decrease in vision bilaterally. Compression of either carotid sinus produced extreme dizziness. The blood pressure and pulse were unchanged except on one occasion when both decreased; on that occasion the dizziness was maximal. Massage of either carotid sinus produced similar results. Angiography was not performed. Lumbar puncture, skull x-rays, EEG, and brain scan were all normal. He was treated with support stockings, Dilantin®, and atropine. The dizziness became very infrequent (and minimal when it did occur). No further episodes of syncope or seizure occurred. He has been followed for one year.

7) A 63-year-old man developed a sudden left hemiparesis and dysarthria two weeks prior to his first visit. During the past year he also had multiple episodes of impaired vision in his right eye. In addition, he had intermittent claudication, diabetes mellitus, hypercholesteremia, urinary frequency, and nocturia. Examination revealed a slight postural hypotension with slight unsteadiness. Compression of neither common carotid artery could be satisfactorily performed because he tensed his neck muscles. Compression of the right carotid sinus produced a "fuzzy" feeling in the head. Compression of the left carotid sinus produced dizziness and falling. His pulse and blood pressure decreased during compression of either carotid sinus. He was not hospitalized. He was treated with support stockings, Dilantin®, and atropine. No further visual or motor symptoms occurred. He has been followed for one year.

Discussion

The foregoing seven cases are illustrative of the varied types of cerebrovascular insufficiency that are included in this series. Thus, Case No. 1 involves angiographically demonstrated complete occlusion of the cervical portion of a carotid artery and reaction to neck compression tests. Case No. 2 concerns angiographically demonstrated multiple vascular anomalies of the circle of

Willis, as well as occlusive disease of the basilar artery, with reaction to neck compression tests. Case No. 3 demonstrates vertebral artery abnormality due either to occlusion or to hypoplasia; reaction to neck compression tests and slight postural hypotension were also present. Case No. 4 includes vertebrobasilar occlusion or hypoplasia along with multiple anomalies of the circle of Willis, with reaction to neck compression tests. Case No. 5 shows unusually severe bilateral hypersensitive carotid sinus syndrome. Case No. 6 illustrates micturition syncope, slight postural hypotension, and reaction to neck compression tests. Case No. 7 demonstrates a classical clinical story of carotid occlusion along with slight postural hypotension and reaction to neck compression tests. Some of these cases were complicated further by cardiac and other illnesses.

The common denominator in these seven cases is cerebrovascular insufficiency; under the treatment described, improvement followed, and has been maintained. The remaining 54 cases in this series are generally similar and, therefore, are not presented in detail.

Transient cerebrovascular insufficiency or transient cerebral ischemia is sometimes classified into two groups—recurrent focal ischemia and systemic hypotension. In recent years there has been a tendency to concentrate therapy on focal ischemic factors such as endarterectomy for carotid occlusive disease. Instead, this presentation emphasizes the importance of the combination of these categories with relatively greater emphasis on the general factors than on the focal because shifting the therapeutic emphasis to these general factors may be more effective and much safer. Ischemic stroke may be defined as the focal result of failure of the total cerebral circulation. That is, total cerebral circulatory failure usually determines *if* ischemia will take place. *Where* the ischemia will take place depends at least in part upon the weakest link—the hypoplasia, stenosis, or occlusion of a given blood vessel. The present approach attempts to improve the general circulation so that even in the presence of apparently severe inadequacy of one or more blood vessels, ischemia will be prevented by a more efficient general collateral circulation.

The method comprises the use of support stockings, carotid sinus therapy, and an anticonvulsant. Support stockings are generally applied first, in order to prevent postural hypotension, whether or not it is manifested under the conditions of the physical examination. This is because under other conditions—such as exposure to heat, external or internal—postural hypotension may occur and may be severe, leading to syncope, seizure, or other symptoms. Additionally, some patients with hypertension have postural hypotension on occasion (whether or not antihypertensive drugs have been used). It is my impression that such patients may have a reduction in their hypertension (and, therefore, in their antihypertensive drug requirements) because of a reduction in the postural hypotension by the support stockings. Whatever the explanation, personal empirical observations of several patients suggest that blood pressure regulation has been distinctly improved, both standing and reclining, when support stockings are added.

Atropine is used for the control of carotid sinus hypersensitivity. Ocular tension and prostatic status are evaluated before it is given. Radiotherapy (1) was added in one patient (No. 5) in whom drug therapy had been inadequate and where it was felt that the hypersensitive carotid sinus was a major part of the problem. It may be noted that thus far, patients on this regimen who also have a history of angina, have shown no significant change in the angina (2).

Anticonvulsant therapy has usually been Dilantin®. A history of paroxysmal episodes suggestive of seizure requires the use of an anticonvulsant. Another indication for anticonvulsant therapy is carotid sinus syndrome or reaction to carotid *artery* compression, especially if obvious seizure activity is evoked by such testing. In addition, one may speculate on the possibility of a "parenchymal factor" in the induction of stroke. It is well known that there are many cases of stroke in which postmortem examination reveals parenchymal disease, i.e., infarction, but no demonstrable significant blood vessel disease (3). Therefore, there seems to be another factor in the production of stroke; perhaps it is parenchymal. Are there, as a result of parenchymal disease, manifestations other than the frank seizure which may respond to anticonvulsant therapy? This is not yet proved. Another indication for the use of Dilantin® derives from the finding of arrhythmias in cases of cerebrovascular insufficiency, by continuous ECG monitoring (4). These arrhythmias have included both bradycardia and tachycardia, and, but for the monitoring, might have been overlooked. Just as atropine may protect the brain from the ischemic effects of some bradycardias, Dilantin® may protect the brain from the ischemic effects of certain arrhythmias and tachycardias. There are enthusiastic proponents of the use of Dilantin® in cardiology in the treatment of arrhythmias and tachycardias (5).

We are concerned with the total collateral circulation—the total ability of the cerebral circulation to maintain adequate perfusion despite focal blood flow failure. The availability of this collateral circulation may be improved through the prevention of general decompensating mechanisms such as postural hypotension, reflex bradycardia and/or hypotension. In addition, protection of the brain parenchyma itself from too ready a tendency to respond with a variety of seizure manifestations may help.

The influence of general factors upon the manifestation of focal factors can be recalled in other situations. The Amytal® test (6) and the hot bath test each produce general stress which may bring out latent focal deficits. However, when these general stress factors are corrected (i.e., removed), the induced focal abnormalities, even if marked, are no longer apparent. In like manner, induced hypotension has been used to evoke transient focal abnormalities which disappear when the systemic blood pressure is only minimally reelevated (7) (8).

In this series of 61 cases are included patients who could have been considered candidates for neck vascular surgery, anticoagulants, vasodilators, or, of course, no treatment at all. Most patients are in their 60's and 70's; treatment in this age group must not be overly vigorous. This method has been well tolerated both in the acute stage of illness and in the long-term follow-up. Now that it has been found applicable and safe over a period of eight years in a heterogeneous group of cases of cerebrovascular insufficiency, I suggest that a controlled study be set up to compare its effectiveness with that of other methods, e.g., surgery, in occlusive disease. It is noted that although angiography was not made mandatory in all cases for the purpose of this initial investigation, it should be made mandatory for a study of comparative treatment.

Summary

1) Support stockings, carotid sinus therapy, and an anticonvulsant have been used, generally together, as a basis of treatment for a variety of cases of cerebrovascular insufficiency, among them patients with occlusive disease of the neck arteries, occlusive disease of the intracranial arteries, hypoplastic arteries; vascular anomalies of the circle of Willis; patients with reaction to compression of the common carotid arteries or of the carotid sinus areas; and patients with postural hypotension.

2) After an eight-year study (a total of 61 patients) with satisfactory results, it is now suggested that this method be compared on a controlled basis with other methods of treatment.

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Monilial Vulvovaginitis Following "The Pill"

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Among the disadvantages of estrogen-progesterone drugs for birth control reported in the literature, monilial vulvovaginitis is a seemingly minor side-effect. However, stubborn recurrences with severe vulval symptoms, mainly the annoying dyspareunia, have to be considered when dealing with this problem.

The group of patients with candidiasis in our Vulvovaginitis Clinic is quite large, not only because of the active obstetrical department at The Mount Sinai Hospital, but because the incidence of monilial infection has increased twofold in the last ten years. When the era of antibiotic therapy began, there was a sudden rise in the incidence of candidiasis, probably because of the change in the bacterial vaginal flora produced. Ordinarily there is an intravaginal competition for growth between the yeasts and other non-bacterial organisms and the pathogenic and nonpathogenic bacteria.

The antibiotics are no longer the primary cause of monilial vulvovaginitis. The higher incidence of candidiasis has a newer source in the oral contraceptives, collectively known as "the Pill."

Since Roch, Pincus, and Garcia introduced in 1956 the estrogen-progesterone drugs as reliable and easily administered birth control therapy, the contraceptive "Pill" became the most accepted and discussed drug in the world. Simultaneously a large number of researchers started to study the negative effects of the pill from all aspects. One aspect of these studies which interested us clinically was the increased incidence of monilial infection in the group of patients using oral contraceptives.

A glucose tolerance test demonstrated a curve simulating a diabetic curve. Therefore, we stress the need to repeat the glucose tolerance test, to study it carefully, and to rule out diabetes mellitus in all chronic, stubborn, recurrent cases of monilial vulvovaginitis. From January 1969 to May 1970 we had seen 574 new patients with monilial vulvovaginitis; 329 were pregnant and 245 nonpregnant. Among the 245 nonpregnant patients with all the clinical symptoms and with the positive Nickerson media, 176 were taking the pill and 69 were using other contraceptive methods. As the table shows, 30.6% of our clinic patients with monilial infection were using the pill. (Table I)

It is well documented that Candida albicans infection appears frequently in pregnant women. Candida requires a large amount of sugar. In pregnancy the vaginal epithelium has a higher glycogen content, providing optimal conditions for monilial growth.

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TABLE I

Total No. of Patients with Monilial Vulvovaginitis	574
No. of Pregnant Patients	329
No. of Nonpregnant Patients	245
No. of Patients Taking the Pill	176 (30.6%)
No. of Patients Using Other Methods	69 (12%)

The change of carbohydrate metabolism in users of oral contraceptives has been described by numerous investigators who agree on a positive increase in blood glucose values in the drug-treated group, with a slower disappearance rate for the glucose. In 1969 Spellacy* published a well written report summarizing all the data available on the effect of oral contraceptives and their component parts upon carbohydrate metabolism. In all pertinent studies there is one common statement, the diabetogenic effect of oral contraceptives is probably due to their estrogen component, and the progesterone part causes no change in carbohydrate metabolism.

It is not within the scope of this paper to deal in physiological detail with the mechanism of the altered carbohydrate metabolism in patients using the pill. All studies suggest that the increased blood glucose values raise the glycogen content of the vaginal epithelium. The greater incidence of monilial vulvovaginitis when using the pill is therefore understandable.

Vulvovaginitis not typical of Candida infection was found recently in a significant group of young patients. Smears and cultures taken in the Nickerson media were positive for Candida albicans. However, the characteristic cheesy, creamy discharge typical of monilia is lacking, and the vagina appears unchanged in most cases, with a scant, more watery discharge. Additionally there is a more serious vulval involvement. The vulva is dry, red, and irritated, or inflamed with edema, and sometimes simple or eczematous intertrigo is present. The subjective symptoms are always severe pruritus vulvae, dysuria, and mainly dyspareunia. This atypical vulvovaginitis resembles diabetic vulvovaginitis.

The patients with this form of vulvovaginitis were found to have been on the pill for a few months or years. Many of them will respond to treatment with the accepted, yet not completely satisfactory, fungicides. But a great number of these patients will have a steady recurrence of moniliasis. However, even a small percentage of the marital partners will be infected by the Candida. After treatment the symptoms will disappear, and a negative culture can be obtained temporarily, then all the subjective and objective symptoms will reappear. There was no relationship between the length of time in the use of the pill and the severity of the symptoms, nor was any improvement found when changing the brand of the pill.

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* Spellacy, W. N.: A Review of Carbohydrate Metabolism in the Oral Contraceptives, Am J Obst & Gynec 104:448, 1969.

Summary

1. Moniliasis is a frequent side-effect of of "the Pill."
2. Pill-induced moniliasis often has a clinical picture differing from the classic form and is stubbornly recurrent.
3. Of the 574 patients with moniliasis, 30.6% were using the pill.

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Agenesis of the Corpus Callosum in a Case of Focal Dermal Hypoplasia

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Of forty-three reported cases of focal dermal hypoplasia (FDH), or Goltz's syndrome, there have been four occurrences each, of mental deficiency (2, 3, 4, 5) and microcephalus, (2, 4, 6, 7), and one of epilepsy (8). To our knowledge, there have been no cases with a demonstrated congenital malformation of the brain. This report considers a typically stigmatized patient in whom, in addition to microcephalus and mental deficiency, agenesis of the corpus callosum was demonstrated by pneumoencephalogram.

Case Report

L.S. (BMH #390341), a negro girl, four years and one month of age, was the product of a full term pregnancy. The birth weight was 5 lb 5 ounces. Multiple congenital anomalies were evident. There is no family history of consanguinity or congenital anomalies. The father, 48 years of age, mother 28, full sister, two, half-sister 6, and half-brothers 11 and 13, are all in good health. The mother is the common parent of all half-siblings.

The patient (Fig. 1) is 40 inches tall, 39½ lb and the head circumference is 17½ in (below the third percentile†). The head is brachycephalic and the neck is short and webbed. The auricles are floppy and seem deficient in cartilage. The teeth are irregularly peg-shaped and unevenly spaced. The left eye is microphthalmic with colobamata of the iris and choroid. With the exception of a widened, partially bifid nail on the right ring finger, the right extremities are normally formed. Both the left arm and left leg are shorter than their right counterparts, and bear the following defects: the third metacarpal has no corresponding digit and ends in a stump; the digit corresponding to the fourth metacarpal is broad, and, by palpation, bears two sets of phalanges; the left foot is short and consists of two broad appendages separated by a median cleft. There is patchy baldness, and the eyebrows are sparse. Hyperkeratosis, atrophy and hypopigmentation, occur in linear streaks over the face, extremities and trunk. Herniation of subcutaneous fat through the dermis is clearly evident over the buttocks and perineum (Fig. 2). There is no spontaneous speech, only single word responses to simple questions, and her total vocabulary numbers 20–30 words. Consideration of her speech lag and general demeanor led to the estimate that she is functioning at approximately an 18-month level, or in the

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† Children's Medical Center, Boston—Anthropometric Growth Chart.

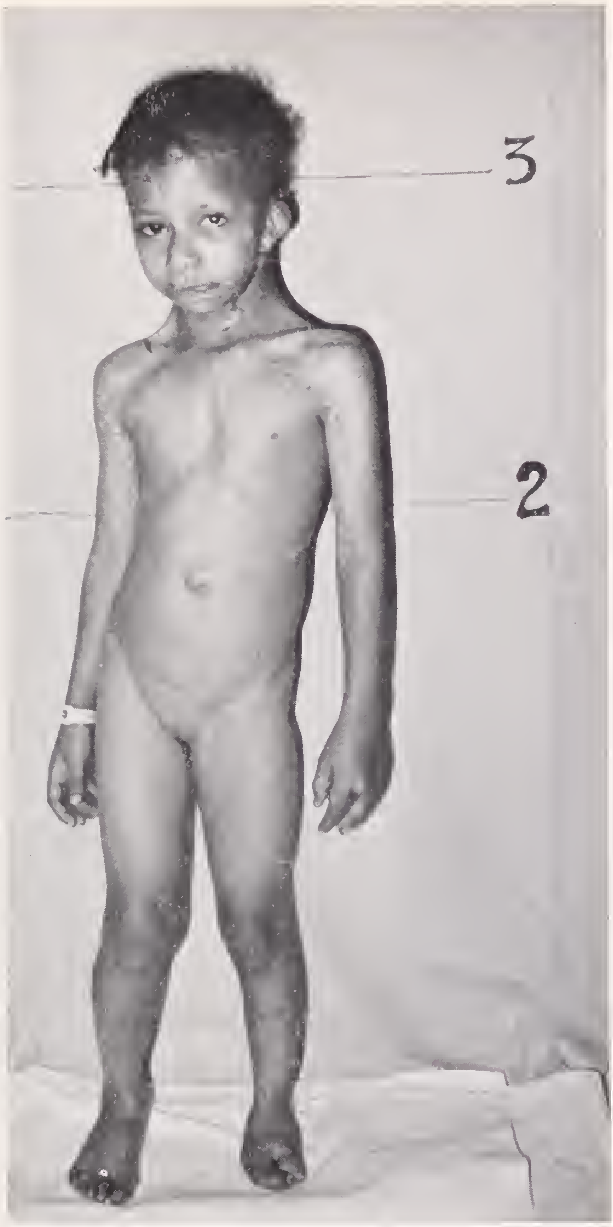

Fig. 1. L.S., age four years, one month.

range of moderate mental deficiency (IQ 35–50). Other than miosis of the defective left eye, there are no focal neurologic deficits.

The CBC and urinalysis were normal. The CSF cell count, protein, and glucose were normal. The serum calcium was 9.8 mg/100cc; inorganic phosphorus, 6.0 mg/100cc; glucose, 70 mg/100cc; blood urea nitrogen (BUN), 16 mg/

100cc; uric acid 4.0 mg/100cc; cholesterol 210 mg/100cc; total protein, 7.3 gm/100cc and albumin, 4.1 gm/100cc. Likewise the alkaline phosphatase, lactic dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), and bilirubin were normal. The EEG was moderately, diffusely abnormal, and no

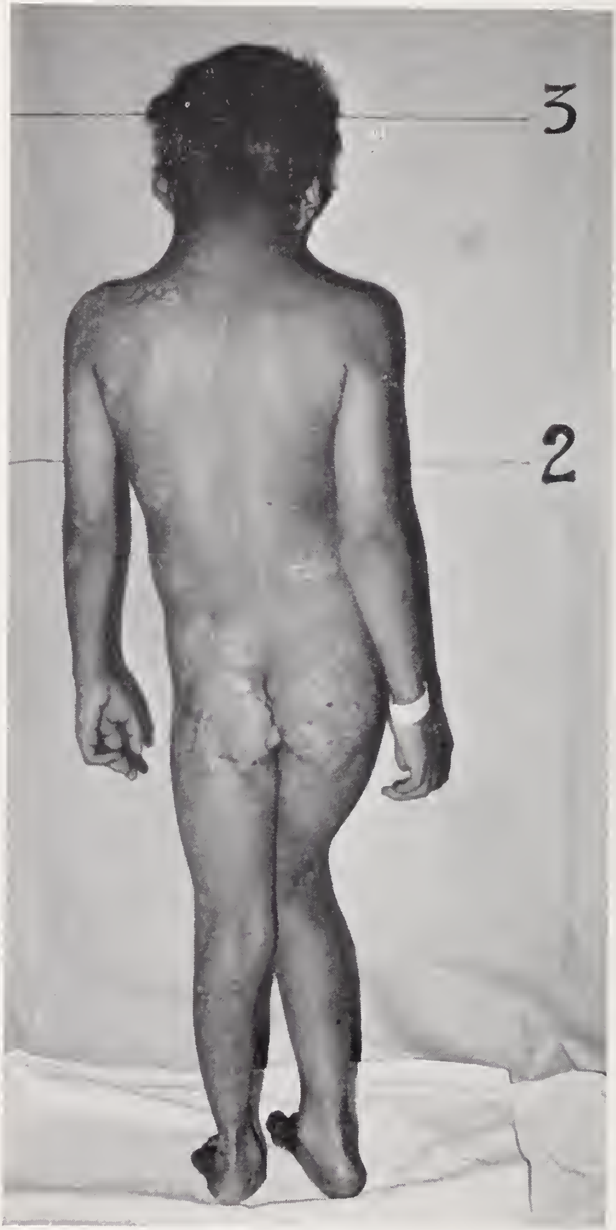

FIG. 2. Note webbed neck; herniation of subcutaneous fat through dermis.

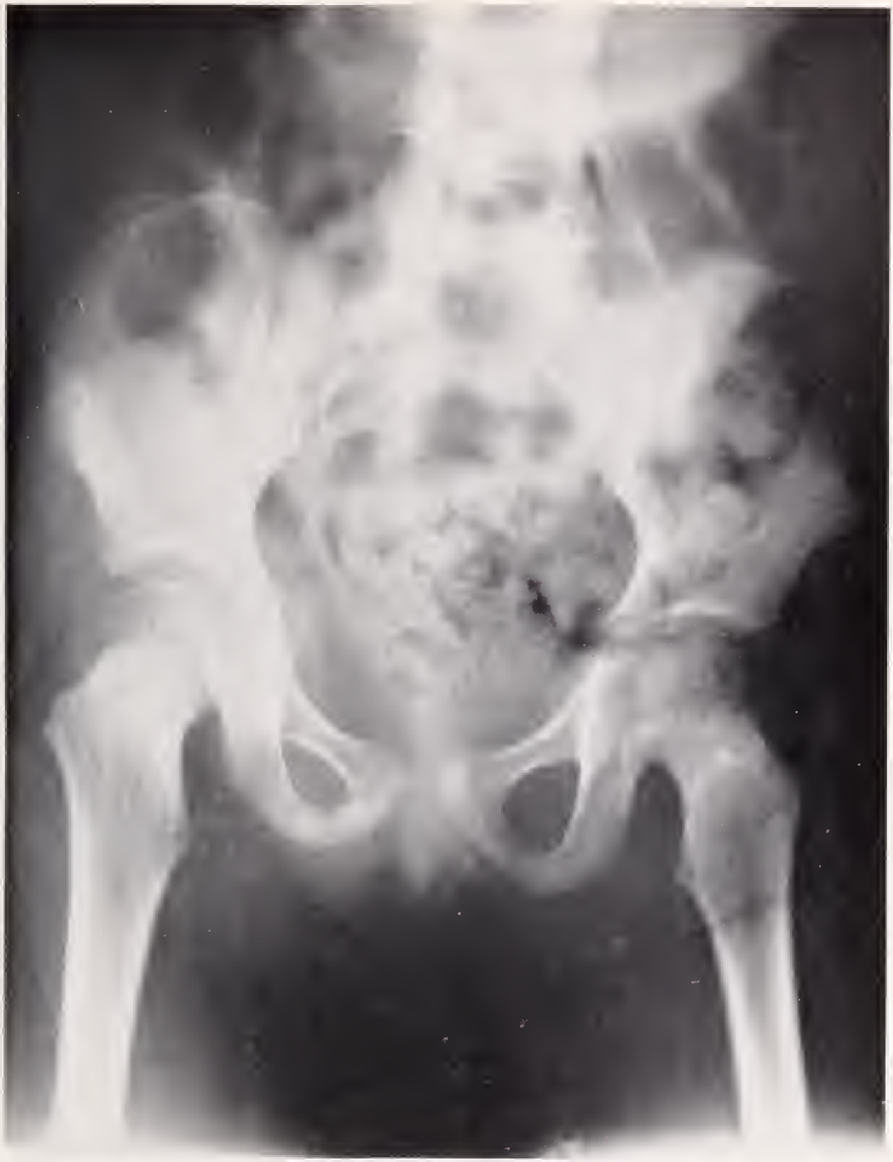

FIG. 3. Bilateral coxa valga.

paroxysmal abnormality was seen. Karyotyping from peripheral blood leukocyte culture revealed a normal, 46 XX chromosome constitution.

X-ray films of the teeth reveal numerous cavities and marked loss of enamel caps. The calvarium is small relative to the face. Films of the spine reveal a spina bifida of C-7. Pelvis studies reveal bilateral coxa valga (Fig. 3) and mild

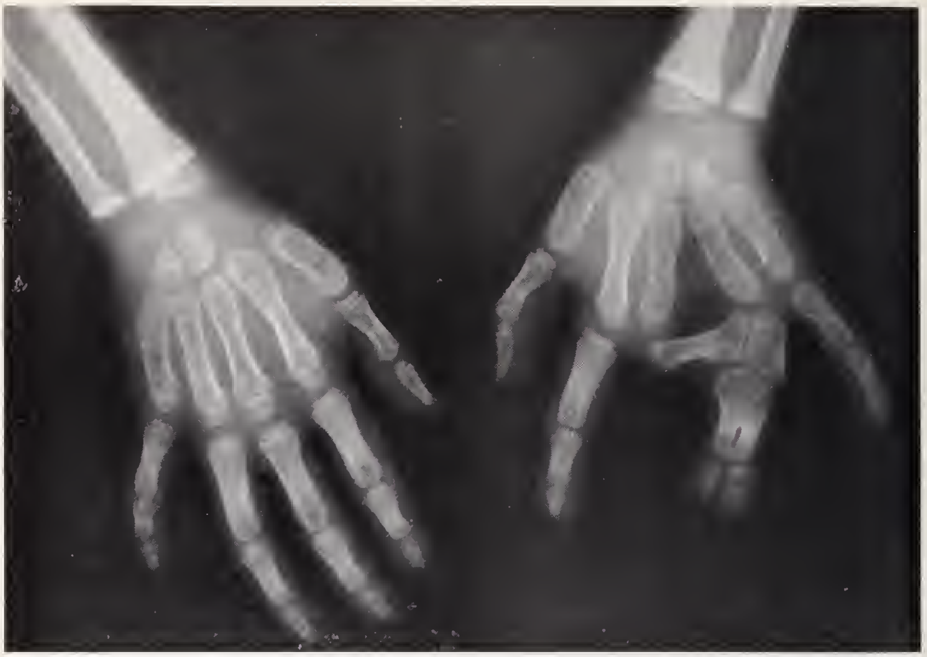

Fig. 4. Anomalous left hand.

osteoporosis. The bones of the right arm and leg and hand and foot, are normal and disclose an appropriate bone age. On the left hand (Fig. 4) there is an anomalous digit corresponding to the fourth metacarpal. It appears to consist of two partially fused proximal phalanges and paired, fused middle and distal phalanges. There is no digit arising from the third metacarpal. X-rays of the left leg reveal a decrease in size of the left tibia and fibula and an absence of the epiphyseal center of the proximal and of the fibula. Except for the calcaneus, talus, and possibly the cuboid, the bones of the left mid-foot are not readily discernible (Fig. 5). There are only two metatarsals, the medial one of which has an attached phalanx. They appear to be separated by a soft tissue cleft.

A lumbar pneumoencephalogram (Fig. 6) was performed, which shows the third ventricle in a high position, separation of the lateral ventricles, and convexity of the medial borders of the lateral ventricles—the "bat-wing" appearance characteristic of agenesis of the corpus callosum. There is also a slight increase in air in the subarachnoid space over the cerebral hemispheres.

Comment

To our knowledge, this is the first report of a definite congenital malformation of the brain in the FDH syndrome. The combination of mental deficiency, subnormal head size, and multiple congenital anomalies led to the suspicion of a

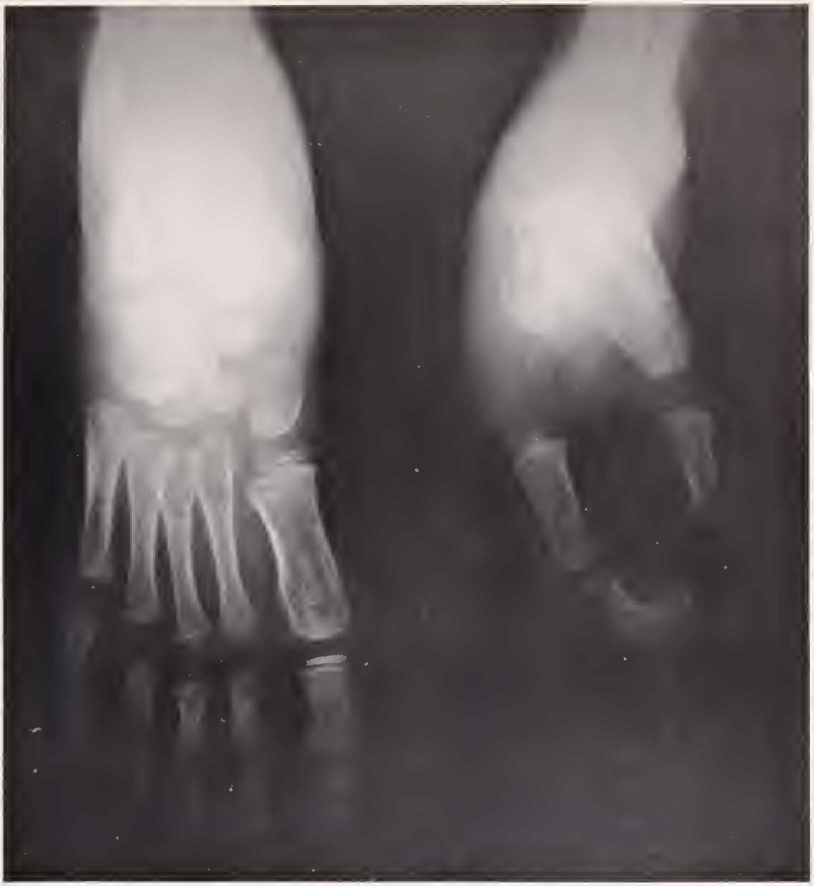

FIG. 5. Anomalous left foot.

malformation of the brain, and the decision to do a pneumoencephalogram. Patients with agenesis of the corpus callosum (9) are usually mentally deficient, often epileptic, and occasionally otherwise malformed. Pathologically, hydrocephalus, porencephaly, microgyria, heterotopias, and other brain anomalies may coexist. Since there appears to be an increased frequency of mental deficiency and, possibly, of other neurologic abnormalities as well, detailed reporting of the mental and neurologic status of patients with the FDH syndrome is urged.

Summary

A negro girl, four years and one month of age, with the focal dermal hypoplasia syndrome, is reported. In addition to microcephaly and mental deficiency, agenesis of the corpus callosum was demonstrated by pneumoencephalogram. This is believed to be the first case of the focal dermal hypoplasia (FDH) syndrome in which a congenital malformation of the brain has been demonstrated.

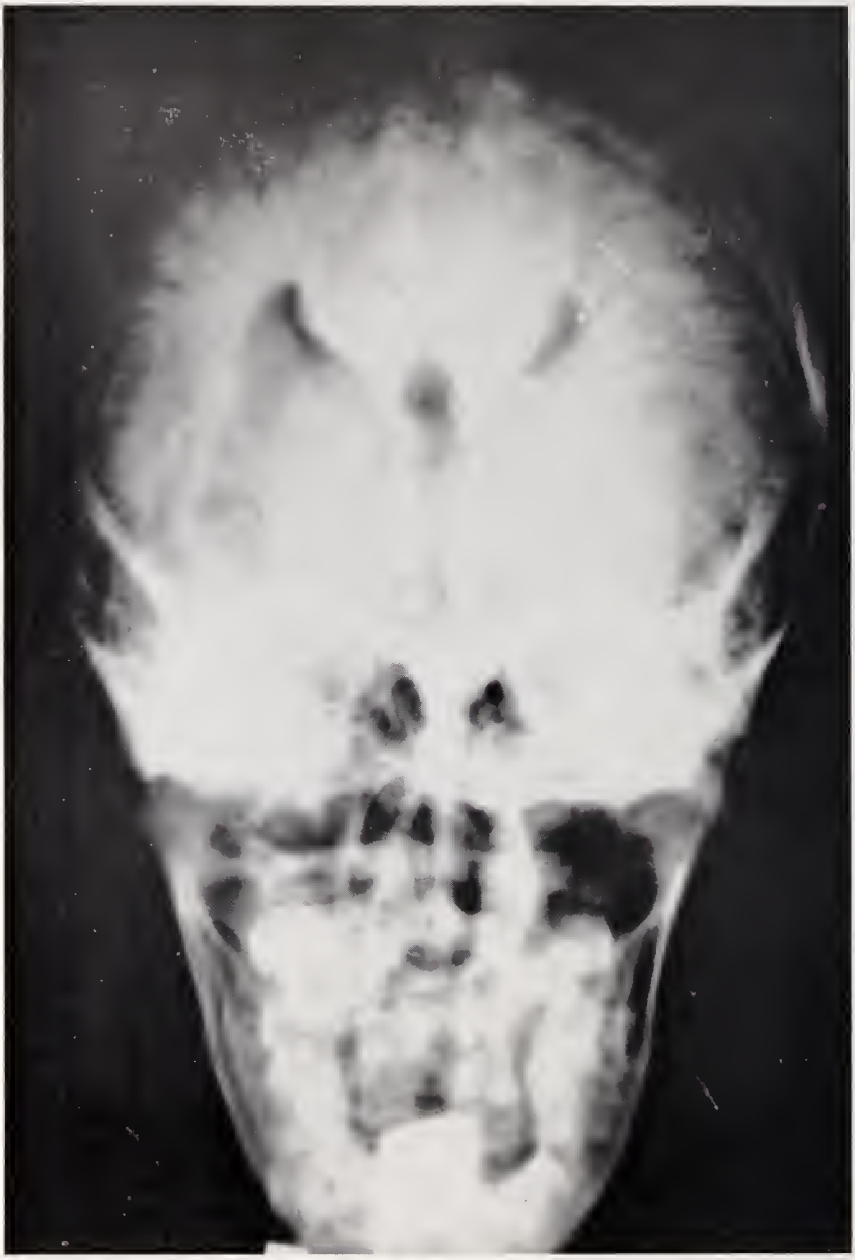

FIG. 6. Pneumoencephalogram. "Bat-wing" configuration characteristic of agenesis of corpus callosum.

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CLINICO-PATHOLOGICAL CONFERENCE

Calcifying Abdominal Masses

Edited by
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A 55-year-old woman, born in the Dominican Republic, was admitted to The City Hospital Center at Elmhurst because of chills, fever, and abdominal pain.

Two years earlier she was hospitalized because of vomiting of coffee ground material. An operation had been performed for hematemesis 13 years previously, but the details were unknown. In the interim, she had two additional episodes of hematemesis which responded to conservative treatment. The patient denied excessive alcoholic intake. Physical examination revealed an alert woman in acute distress. The respirations were 120 per minute, temperature 99.2°F, pulse 106 per minute, and blood pressure 90/60 mm Hg. The mucosae were pale. The heart and lungs were normal. The liver was palpated 4 fingerbreadths below the right costal margin, and there were two epigastric abdominal masses, each 8 to 9 cm in diameter. The rectal examination was unremarkable. The hemoglobin was 6.8 G%, hematocrit 21%, WBC 8,100/mm^3 with a shift to the left. The blood sugar was 98 mg%, BUN 23 mg%, albumin 4.1 G%, globulin 4.0 G%, and alkaline phosphatase activity 1.6 King Armstrong units. A serologic test for syphilis was nonreactive. X-ray film of the abdomen revealed numerous spherical calcified cystic masses, located mainly in the upper abdomen. An upper gastrointestinal series showed a filling defect of the fundus and pars media of the stomach. An x-ray film of the chest disclosed a rounded density at the base of the right lung near the cardiac border. Several stool examinations were positive for occult blood. She received multiple blood transfusions and during the second week of hospitalization an abdominal operation was performed.

For the next ten months she was followed as an outpatient because of brown serous drainage from the surgical wound. She stated she had been hospitalized during the preceding year because of gastrointestinal bleeding and had received blood transfusions. On admission to The City Hospital Center at Elmhurst, her temperature was 102.5°F, pulse rate 102, respirations 26, blood pressure 112/70. She was pale and appeared chronically ill. The abdomen was distended and a draining fistula was noted in the epigastrium. A large, firm mass was palpated in the left hypochondrium. The liver edge was felt 4 fingerbreadths below the right costal margin and extended across the midline.

The hemoglobin was 9.1 G%, WBC 5,100 with 78% neutrophils, 1% band forms, 20% lymphocytes and 1% monocytes. The urine contained 6 to 8 white cells per high power field, and a trace of albumin. The serum electrolytes were normal. The serum amylase was 46 Somogyi units, serum albumin 4.0 G%, alpha$_1$ globulin 0.4 G%, alpha$_2$ globulin 0.9 G%, beta globulin 1.1 G%, and

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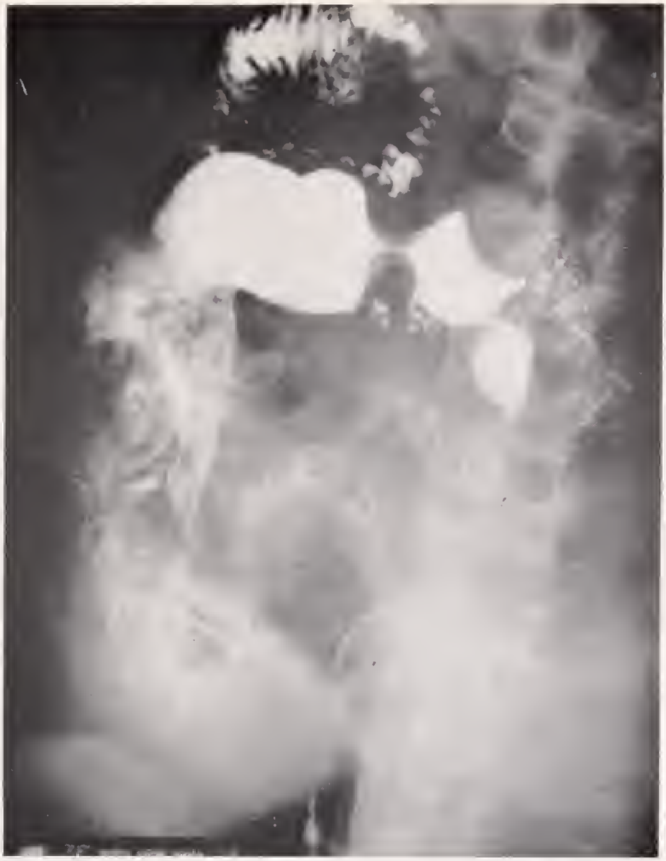

X-ray-1. Upper GI series performed four years prior to admission, showing multiple spherical calcific densities having a cyst-like appearance, and a filling defect in the fundic region of the stomach.

gamma globulin 1.8 G%. The BUN was 9 mg% and serum calcium 9.5 mg%. A scout film of the abdomen showed multiple calcified cysts throughout the liver. An x-ray film of the chest showed a 3 cm nodule in the right middle lobe and a smaller lesion in the lingular segment of the left upper lobe.

She received blood transfusions, and gastroscopy revealed an ulceration over an elevated zone of mucosa on the greater curvature of the stomach. Blood was found in multiple stool examinations. An operation was performed on the 23rd hospital day.

Dr. J. D. Waye:* This is a case of a woman from Santo Domingo with large abdominal calcifications and intrapulmonary masses.

Of a large number of parasitic diseases that may produce calcified lesions, one of the first to consider is schistosomiasis. Calcifications in schistosomiasis, however, are usually small, and are found in walls of the bladder or ureters.

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Another parasitic disease that may lead to calcification is trichinosis. There also are very small flecks of calcium which are usually seen within the muscles. In cysticerosis the calcifications again are usually less than one centimeter in size. Finally, echinococcosis is a parasitic disease that we all know is associated with calcified unilocular and multilocular cysts. The initial infection in echinococcus disease is frequently unnoticed; however, patients eventually develop abdominal pain, vomiting, and symptoms related to pressure of the cysts on associated structures. There may be necrosis of the involved tissue and sometimes leaking of the cyst fluid which produces severe febrile and sometimes fatal hypersensitivity reactions. Echinococcus granulosus, the smallest tapeworm of medical significance, grows to about nine centimeters in size in the intestine of the dog, but may also parasitize foxes, wolves, and coyotes. The eggs are passed by the dog, ingested by cows, and thence by man. The eggs hatch in the gut, pass through the intestinal wall, and circulate throughout the body to be filtered out in the liver and the lungs. The hydatid cyst stage then ensues, which is not the end stage of the disease except in man. In the complete cycle, the hydatid cysts develop in the cow. When the cow's intestine or other parts of the cow are eaten by dogs, these cysts break down and reinfect the dog. Since dogs do not usually eat men, the end stage of the hydatid disease occurs in man.

Echinococcus multilocularis develops by budding rather than by forming cysts. There is no limiting membrane or capsule in the intermediate host, and they grow by extension, simulating malignant disease.

Heavy infestations occur mainly in sheep- and cattle-raising areas, especially in South America, Australia, New Zealand, South Africa, Central and Northern Europe, and the Mediterranean area of Asia and Africa. Clinically, eosinophilia is not marked. The liver is affected in about 75 percent of the cases. The lungs are involved in about four to 15 percent of the cases. Some areas of calcification are apparent in about 35 percent of the cases. The chest x-ray photograph of this patient showed rounded areas without calcification. In the abdomen, however, there were deposits of calcium. Since she had intra-abdominal calcifications, pulmonic disease without calcification, and no eosinophilia, I must consider that she had echinococcal disease. Santo Domingo is not an endemic area for echinococcal disease, but she may have acquired her disease elsewhere.

The calcifications found in echinococcal disease are thin and appear as eggshells. They may be much larger than calcifications seen in this case. An interesting feature of echinococcal disease in the lungs is that the cysts usually grow about three times as fast as the cyst in the abdomen. This is believed to be due to the decreased resistance of the lung. This patient's pulmonary cysts were much smaller than her abdominal cysts. A disturbing feature of this case was that when she came to our hospital after having had her disease for a number of years, the cysts were much larger. Characteristically, once the cysts calcify they do not enlarge. In addition, draining sinuses are not a feature of echinococcal disease.

Two types of processes are believed to result in clacifications within the body.

Dystrophic calcification occurs in dead or degenerating tissues, is the most frequent cause of calcification, and has no particular localization. It is thought that the dead or dying tissue produces a change in pH toward the alkaline side and allows precipitation of calcium salts. Such calcifications occur in tuberculous lesions, arteriosclerotic plaques, degenerating tumors, and necrotic fat. Metastatic calcification occurs in hypercalcemic syndromes such as with hypervitamosis D and hyperparathyroidism.

If we consider this woman's disease in relation to the organs involved, at the very top of the list is echinococcal disease and calcifications within liver abscess. Calcification in the liver may also be seen with metastatic carcinomas of the liver, intrahepatic gallstones, tuberculous granulomas and hepatomas. Hemangiomas also may calcify in the same manner as seen in blood vessels throughout the body. Calcifications within the gallbladder include stones, and are rarely within the wall of the organ itself. I do not think we have to be concerned about splenic calcification except for the fact that cysts of the spleen may calcify. With cystic disease of liver and spleen, cysts may also occur in the pancreas but are not usually associated with pulmonary cysts. Calcifications in the urinary tract are found in malignant tumors. Lymph nodes in the abdominal cavity may calcify, but they do not usually achieve this size, and the calcium is deposited in a reticular pattern within the inner portion of the lymph node and is less dense than the outer portion.

Hemangiomatous transformation of the mesenteric veins may lead to thrombus formation and calcium may be laid down in the form of phleboliths. Calcified fibroids need not be considered in the present case, since the location was not typical. Other causes for calcification in the intestinal tract are foreign bodies, tumors, leiomyomas and leiomyosarcomas. If extra visceral calcifications are considered, the most probable etiologies would be echinococcus, calcified parasites such as the guinea worm and abscess.

Prompted by the history of abdominal masses, fever, bleeding and the gastroscopic findings, I also considered that this woman had neoplasm. Leiomyomas, leiomyosarcomas, fibromas, fibrosarcomas, ganglioneuromas, hemangiomas, mesenteric cysts, neuroblastoma, adrenal carcinoma, pheochromocytomas, teratomas in the liver, hepatomas, hemangiomas, and metastatic tumors are but a few of the neoplasms that may contain calcium deposits.

One interesting facet of her history was that she had abdominal calcifications and a history suggesting neoplasia, but she also had upper gastrointestinal bleeding. Certainly echinococcal disease is not usually associated with gastrointestinal bleeding. Diseases of the stomach that cause hematemesis include acute and chronic gastritis, which is not probable in this woman. She did not receive any drugs that are known to cause upper gastrointestinal bleeding. These include aspirin, phenylbutazone, steroids, anticoagulants, alcohol, and reserpine. The patient did not have pseudoxanthoma elasticum which is also associated with degeneration of the elastic lamina of the retina, producing angioid streaks.

Hemangiomas may calcify but do not produce a florid clinical picture. Another disease that may show symptoms similar to gastric tumors are lymphomas. They may ulcerate, infiltrate, and produce a polypoid lesion of the stomach. Lymphomas may calcify but they do not usually produce round, cannonball calcifications. Leukemia is another neoplasm that may cause hematemesis, but need not be considered in this woman.

I mentioned carcinoma earlier in the discussion, and considered the various tumors that calcify. Bockus states that the presence of an epigastric mass in a person who does not appear ill but has symptoms of long duration, should arouse a suspicion of sarcoma. The gastroscopist described a submucosal tumor with ulceration which suggested a leiomyoma or leiomyosarcoma. There have been well over 1,000 documented cases of smooth muscle tumors of the intestinal tract. In fact, 2.5 percent of all gastric tumors are of smooth muscle origin. Forty percent of patients with leiomyomas, and 30 percent of the patients with leiomyosarcomas have a history of longer than 12 months' duration of the disease before diagnosis. Sometimes the onset of symptoms may date back 25 years, and it has been suggested that a leiomyoma may degenerate into a leiomyosarcoma with time. Leiomyosarcoma is a tumor of low malignancy. The liver is the most common site of metastases, and the next most common site is the lung; frequently they also metastasize to local mesenteric and omental nodes.

This woman most likely had a tumor that calcified and which was associated with upper gastrointestinal hemorrhage. The gastroscopic picture was consistent with submucosal lesions such as leiomyoma or possibly leiomyosarcoma. Because of her long history, I think that this patient probably had a leiomyosarcoma.

Question: Were cystic calcifications all in the stomach?

Dr. Waye: Most of them were in the liver. Some of them were in the gastric wall, and some of them appeared to be in the mesenteric space and mesenteric nodes.

Dr. I. S. Schwartz:* When the laparotomy was performed the surgeon found fibrous adhesions in the abdomen and multiple tumor-like masses involving the anterior and posterior walls of the stomach, extending from the esophagocardiac region to the pylorus. In addition, masses were found in both lobes of the liver. One of the larger masses was ulcerated and involved, in continuity, both the lesser curvature of the stomach and the left lobe of the liver. The pathologic specimen consisted of a tumor nodule with cystic areas and multiple zones of hemorrhage. Seen in cross-section the tumor had a yellow to tan appearance with a few white streaks coursing through it (Fig. 1). There was a sharp demarcation between the nodules and overlying gastric mucosa.

An x-ray film of the specimen showed four or five areas of calcific deposition within the mass. There were zones of hemorrhage and irregular strands of fibrous connective tissue (Fig. 2). On microscopic examination the tumor was moderately cellular and there was no evidence of gland formation. The tumor was of mesenchymal origin and was characterized by ovoid to elongated cells

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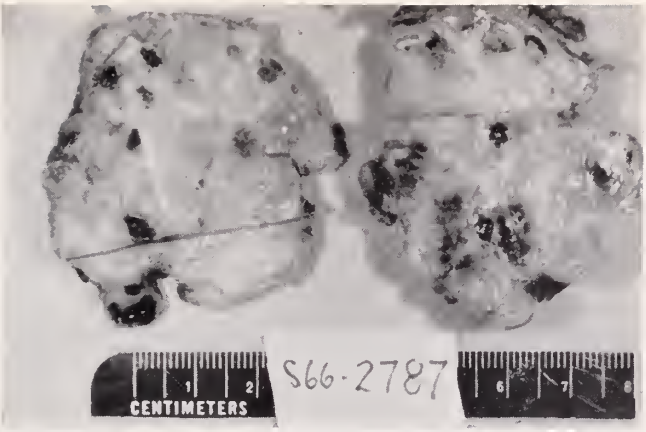

Fig. 1. Gross appearance of cut surface of tumor removed from the stomach in 1966. The mass contained small cystic areas and foci of hemorrhage and calcification. A portion of overlying gastric mucosa is present at the superior portion of section on the right.

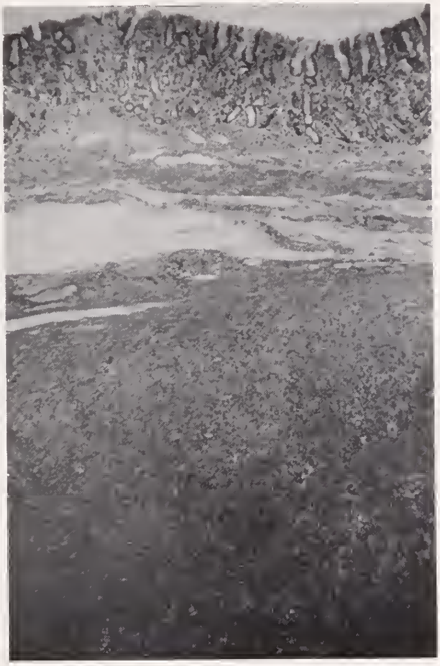

Fig. 2. Low power microscopic view of tumor which appears circumscribed lies subjacent to an intact mucosa.

with uniform nuclei. In some areas there was a streaming or interlacing pattern. Mitotic figures were few (Fig. 3). An unusual finding in the lesion were giant tumor cells (Fig. 4).

The presence of bizarre cells in a tumor of smooth muscle origin does not necessarily indicate it is malignant. In some areas the tumor assumed an orga-

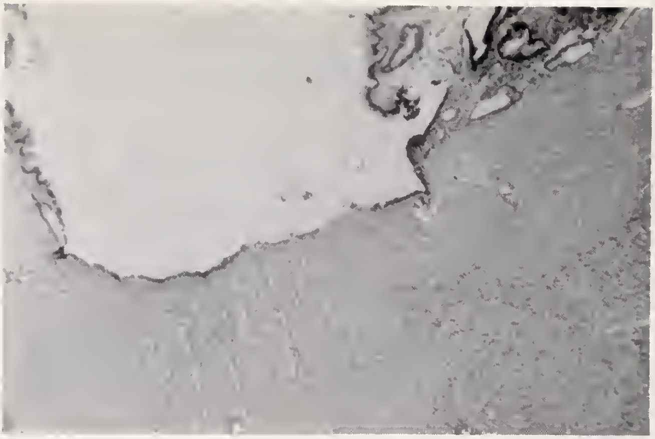

Fig. 3. Microscopic view showing ulceration of gastric mucosa overlying tumor (low power).

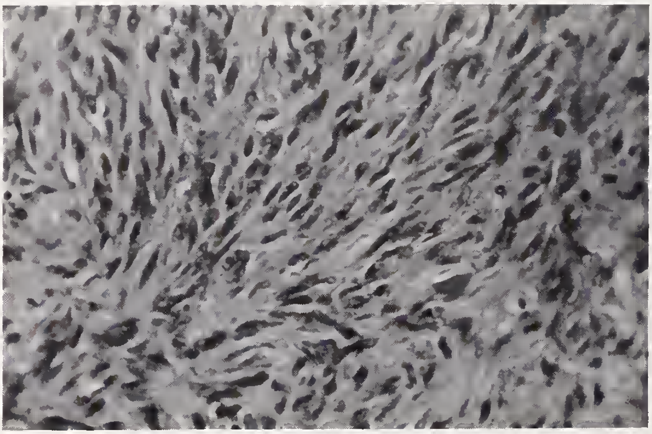

Fig. 4. Histologic appearance of the tumor which is composed of elongated cells with spindle-shaped nuclei. Mitotic figures are extremely rare.

noid appearance in that the cells clustered into tight compact masses separated by connective tissue strands. This has been described in smooth muscle tumors, although if it is found in large numbers the possibility of other neoplasms, such as paragangliomas must be considered.

A stain for connective tissue demonstrated that collagen was almost exclusively limited to the perivascular areas and extended out as delicate fibrous septae.

The area of mucosal ulceration showed regeneration of epithelium over a chronic ulcer (Fig. 5).

During an operation two years prior to admission, a marsupialization was performed on one of the cysts. The pathologic specimen was described as cystic masses containing hemorrhagic fluid. The wall was composed of dense fibrous

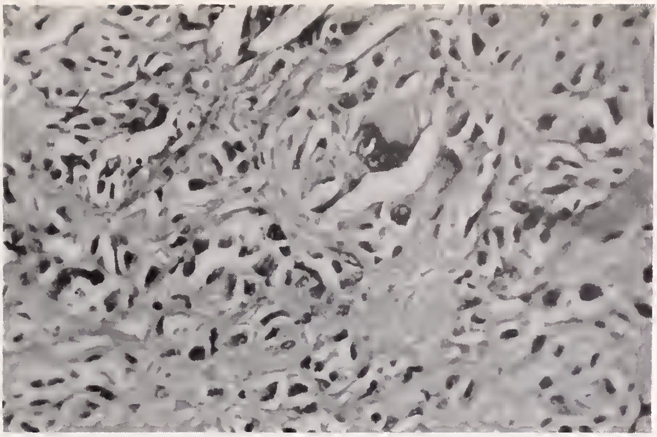

Fig. 5. Microscopic field demonstrating one of the few bizarre giant tumor cells and a less regular pattern than seen in most areas of the neoplasm.

tissue which was partially calcified, and which was lined in part by fibrin and old blood. Histologically they did not resemble echinococcal cysts, and an extensive search failed to reveal hooklets or scolices.

In a portion of the wall of one of the cystic masses were tumor cells in direct apposition to blood-filled spaces. The cells were elongated or spindle-shaped, and tended to coalesce into tight clumps which lay in blood-filled spaces. A lymph node included in the specimen also revealed clusters of tumor cells. A diagnosis of a leiomyosarcoma was made.

In a collected series of 651 cystic tumors from Columbia University and published by the Armed Forces Institute of Pathology, leiomyoma was the most common benign tumor, and carcinoma was the most common malignant tumor, representing almost three-quarters of the cases. Primary lymphoma of the stomach accounted for seven percent of the cases; next was leiomyosarcoma.

It should be remembered that it is extremely difficult to assess the malignancy of smooth muscle neoplasms of the stomach and, therefore, some authors, notably Lumb, simply use the term "smooth muscle tumor." If, however, there is abundant mitotic activity, one may suspect that the lesion represents a sarcoma. An extremely cystic tumor of the stomach which grew into the peritoneal space as a large cystic mass and ulcerated the mucosa of the stomach, was described in the *Journal of Pathology and Bacteriology.*

Our patient had a slow-growing tumor and despite the fact that it has metastasized, she is presently at home with a continual draining sinus, but caring for herself. Her chest film has not changed and still shows at least two metastatic foci.

Final Diagnosis:

LEIOMYOSARCOMA (LOW GRADE) OF STOMACH, WITH METASTASES TO OMENTUM, PERITONEUM, LIVER (AND LUNG, CLINICALLY).

Acknowledgment

The participants wish to express their appreciation to Dr. Arnold Statsinger, Director of Laboratories of Wyckoff Heights Hospital, who originally diagnosed this case and provided pertinent clinical data and pathological material.

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RADIOLOGICAL NOTES

CLAUDE BLOCH, M.D. AND HARVEY M. PECK, M.D., Co-Editors

CASE NO. 342

This asymptomatic 46-year-old man was noted to have an abnormal chest x-ray and was referred for diagnostic evaluation. Examination of the chest revealed a homogeneous density measuring 4 × 2 cm in diameter within the right paratracheal region (Fig. 1A).

In the lordotic view, the outer contour of the mass was noted to be sharp inferiorly (Fig. 1B). It obliterated the outline of the ascending aorta but did not displace the trachea. In the lateral view of the chest, the mass was noted to be situated anteriorly in the retromanubrial region just above the manubriogladiolar articulation (Fig. 2A). It was sharply demarcated inferiorly and posteriorly, and was lobulated. Its anterior contour faded into the margin of the sternum. Tomographic sections through the level of the tumor, revealed a traget-like mottled calcification within the lower portion of the mass (Fig. 2B). The patient had no signs or symptoms of myasthenia gravis. The remainder of the physical examination was entirely normal. The diagnosis of an anterior mediastinal tumor was made. The possibility of a chondroma arising from the sternum was suggested, but the possibility of a thymoma could not be excluded. At operation, the mass was seen to arise from the under surface of the right half of the manubrium. It appeared lobulated, yellowish, and soft, but well encapsulated. Pathology report was of a chondromatous tumor with morphological features which indicate a low grade of histological malignancy. The patient made an uneventful postoperative recovery.

Discussion

Chondromatous tumors of the sternum are rare but usually present as asymptomatic mass lesions on routine chest x-rays. This was the case in the patient presented. The mass is usually sharply delineated and lobulated, and cannot be separated from the posterior portion of the sternum. Calcifications are usually coarse and well circumscribed. Differential diagnosis rests between thymoma, dermoid cysts, and other anterior mediastinal tumors such as lymphomas.

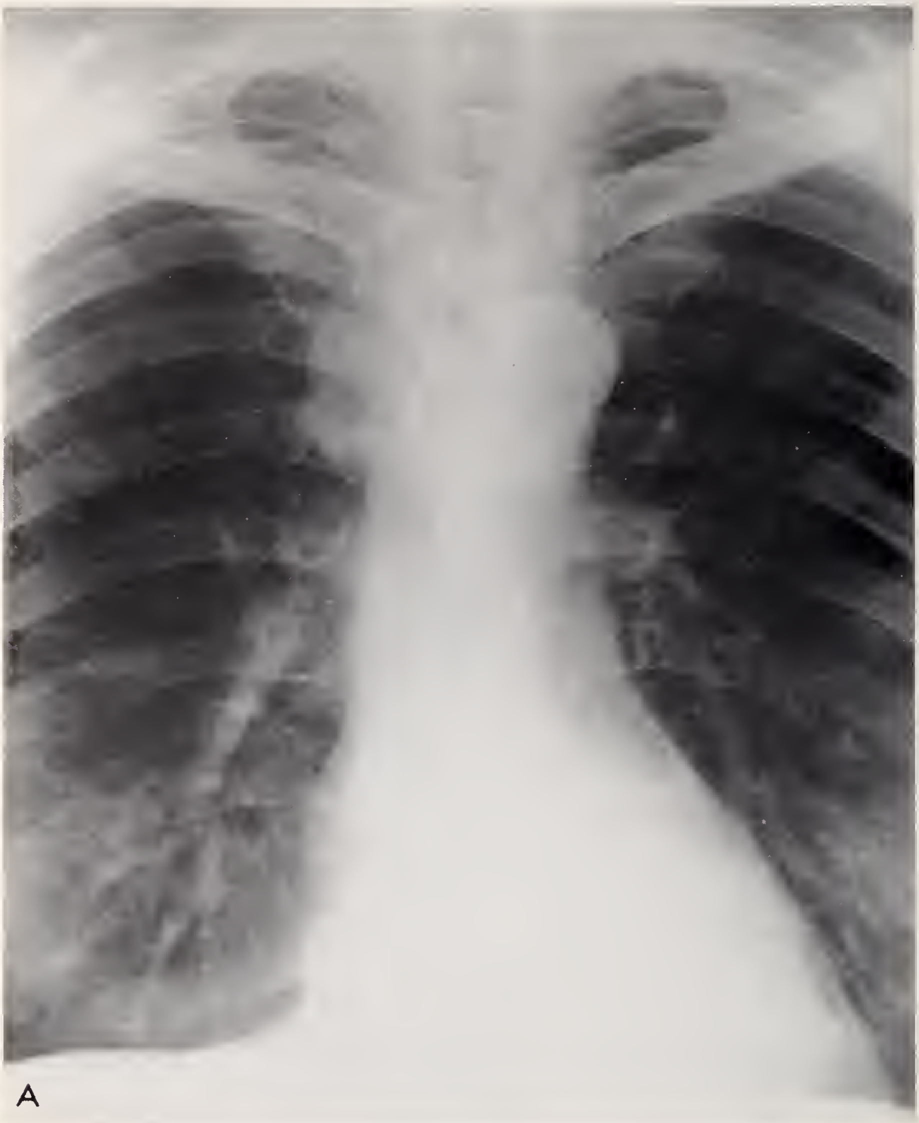
Acknowledgment

The editors wish to thank Dr. S. Theodore Wild and Dr. Paul A. Kirschner for permission to publish this case.

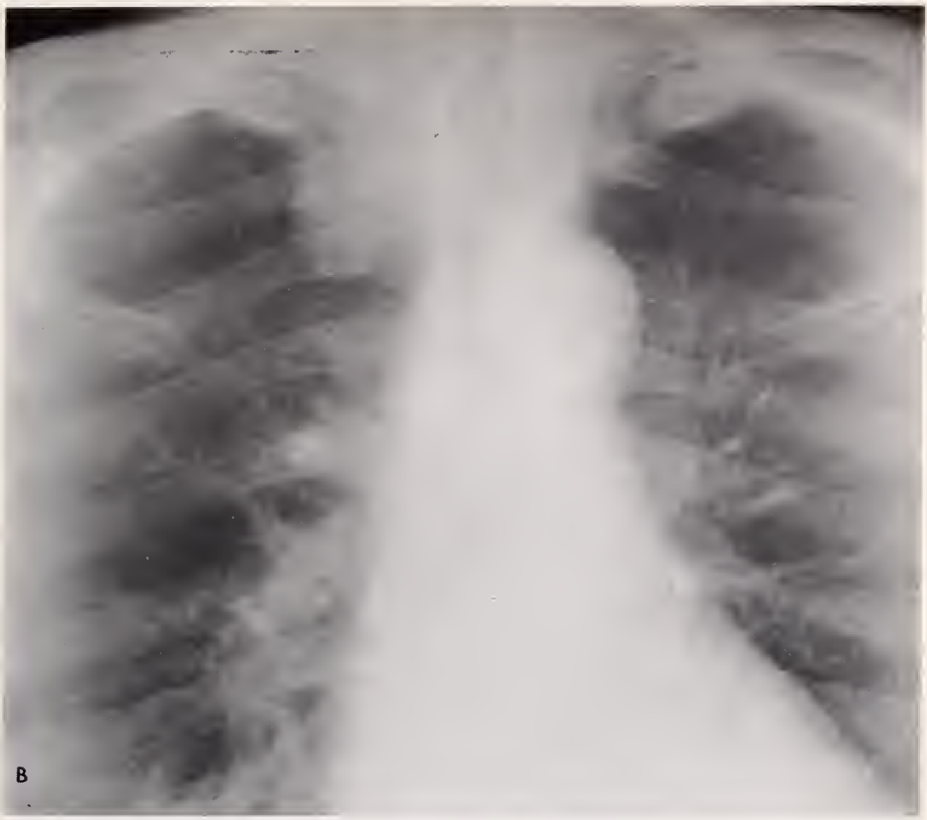
Case Report: CHONDROSARCOMA OF THE STERNUM

CASE NO. 343

This 70-year-old woman was admitted to the hospital because of a history of heartburn, dysphagia, and some weight loss. Symptoms had been present for the last three years with some progression in the last few months. The patient was



Case No. 342, Fig. 1A Postero-anterior view of the chest reveals a homogeneous density measuring 4 × 2 cm in diameter, in the right paratracheal region. It obliterates the outline of the ascending aorta. The trachea is not displaced; the heart and mediastinum are otherwise normal.

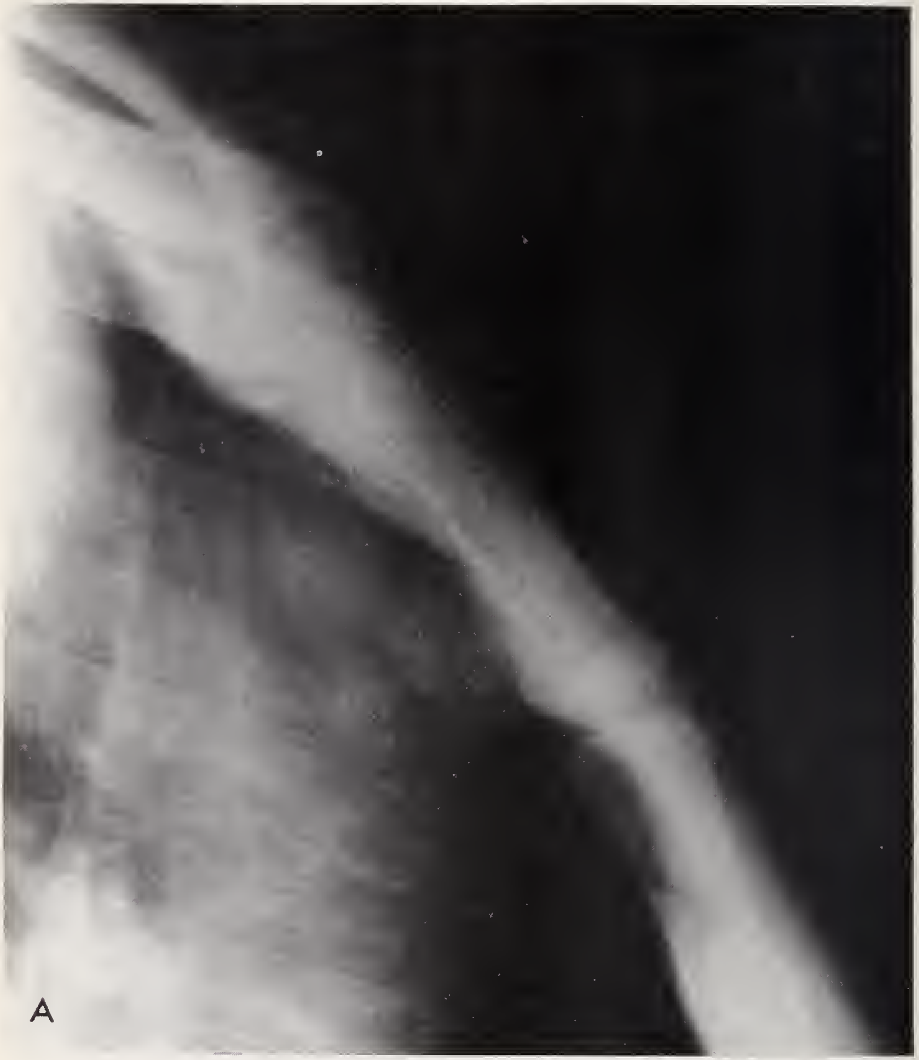


Case No. 342, Fig. 1B Apical lordotic view of the chest reveals the mass to be sharply delineated inferiorly and laterally.

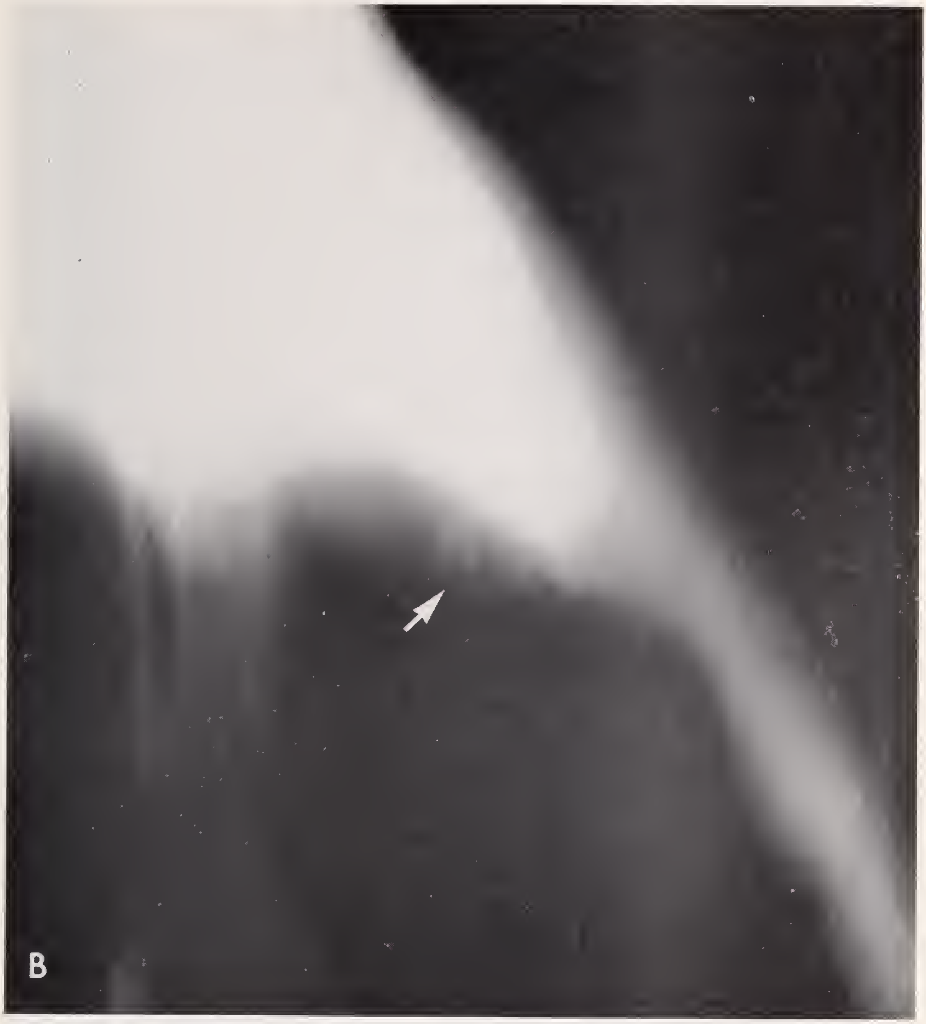
more symptomatic at night, when lying on her back in bed. These symptoms were somewhat relieved by sitting in bed, and with ingestion of antispasmotics and antacids. Because of the progressive nature of these symptoms, an upper gastrointestinal tract examination and chest x-ray where performed, which revealed the following: In the frontal view of the chest, there was a mass in the right cardiophrenic region; a faint calcification was noted within the outer rim of this lesion; within the center of the mass there was evidence of air (Fig. 1A); this pathologic process in the right chest was confirmed in the right lateral view (Fig. 1B), where the calcification was noted to be posteriorly located, with the loculated gas shadow anterior to it.

A barium meal examination was then performed, which confirmed the initial impression of a large hiatus hernia. The air seen in the chest x-ray outlined the fundus of the stomach. The calcifications were noted to be above the herniated portion of the stomach at the distal most portion of the esophagus near the cardia (Fig. 2).

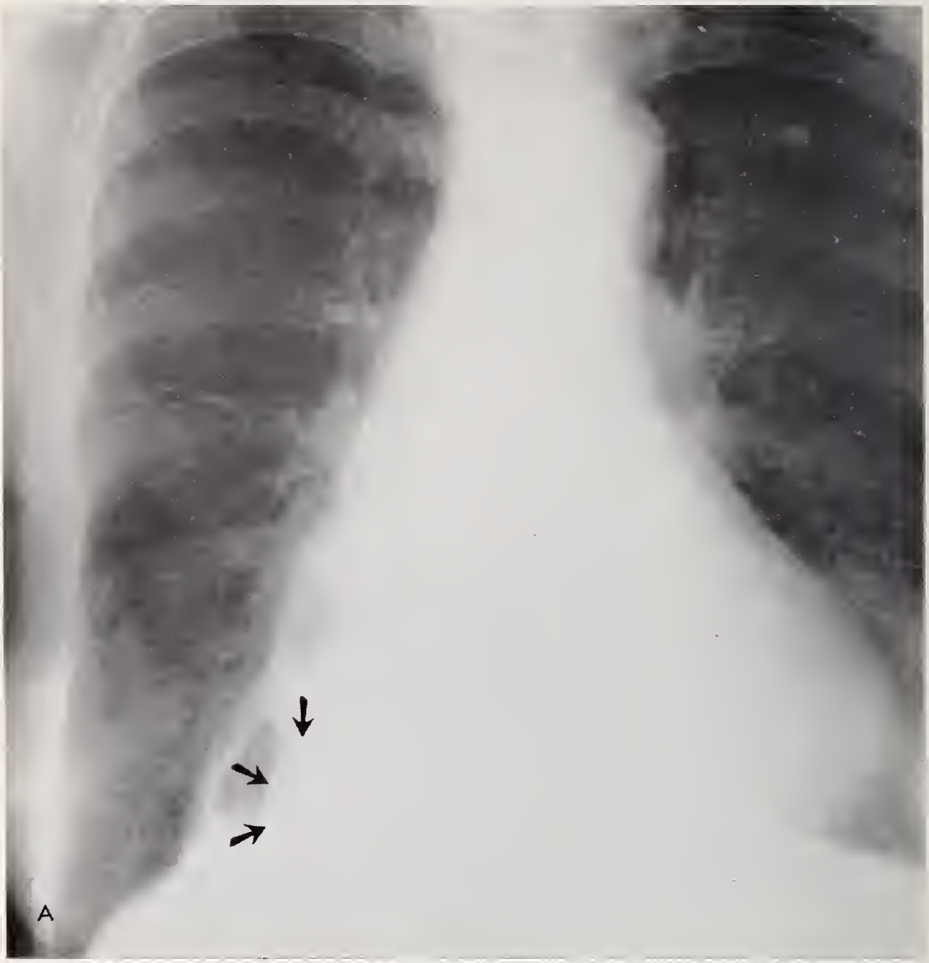
Because of the findings of a calcified mass in the distal esophagus, the patient



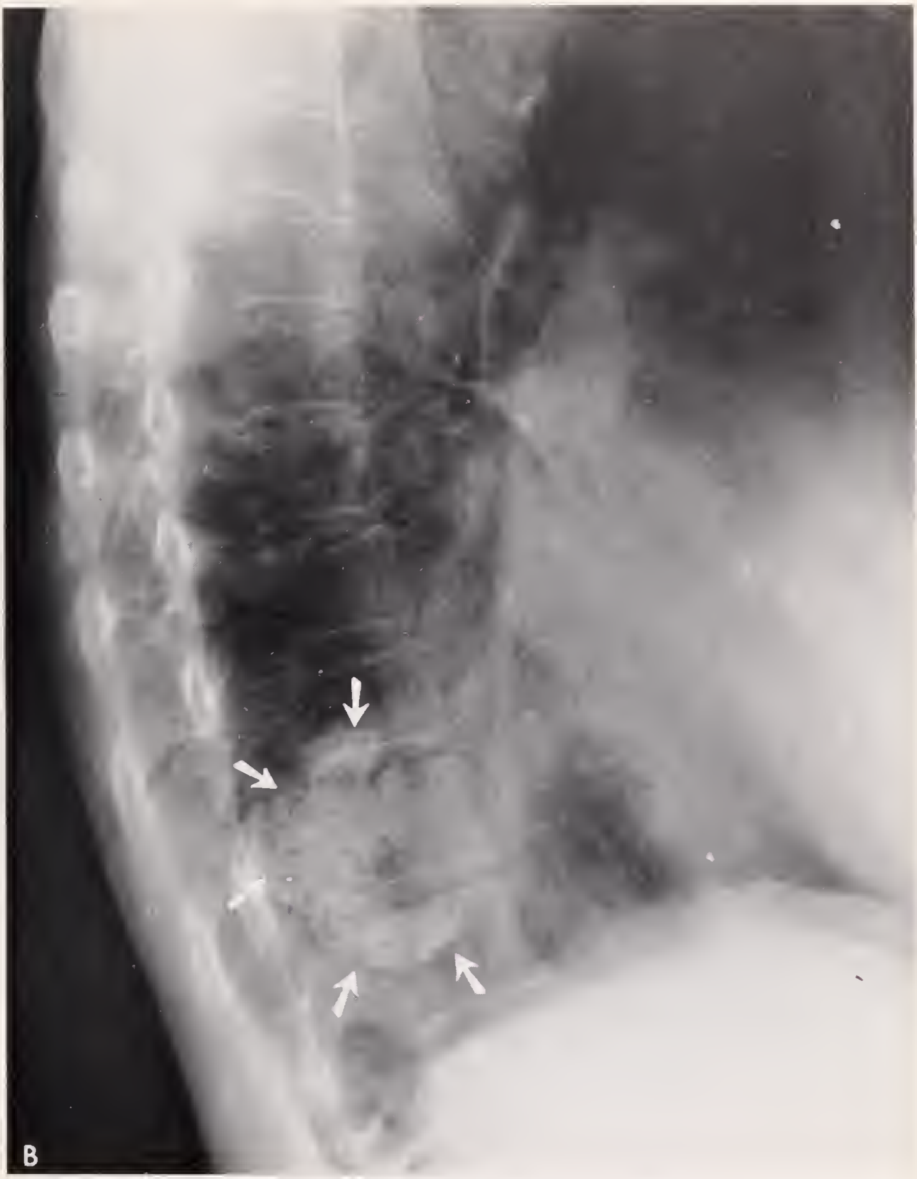
Case No. 342, Fig. 2A In the lateral Bucky view of the chest, the mass is located anteriorly in the retromanubrial region, just above the manubriogladiolar articulation. Its inferior and posterior margins are sharply outlined, but lobulated. It fades off anteriorly into the sternum. A hazy area of increased density is noted within its anterior inferior portion substernally. This has the appearance of calcifications. No bone destruction is identified.



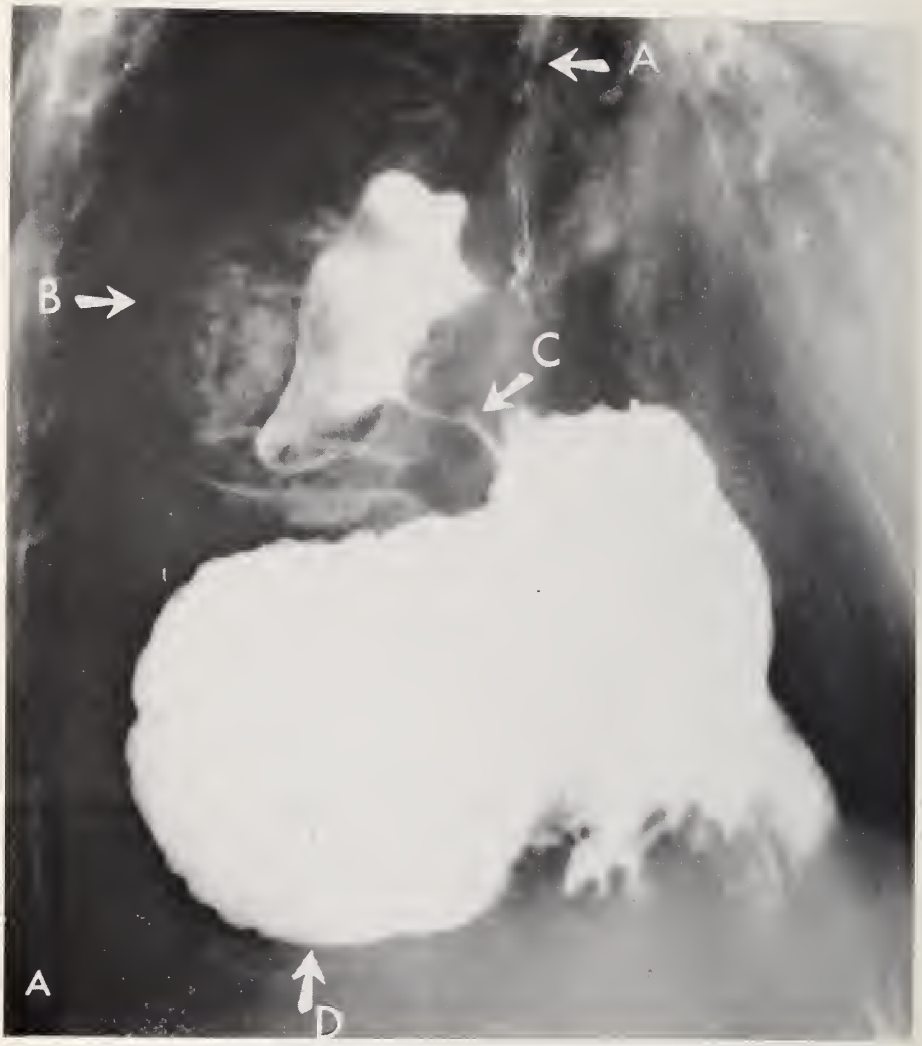
Case No. 342, Fig. 2B Tomographic section through the level of the tumor confirms the presence of a coarse calcification within the lower portion of the mass. It has a rounded outer outline and an inner punctate appearance suggesting a "target" configuration (arrow).



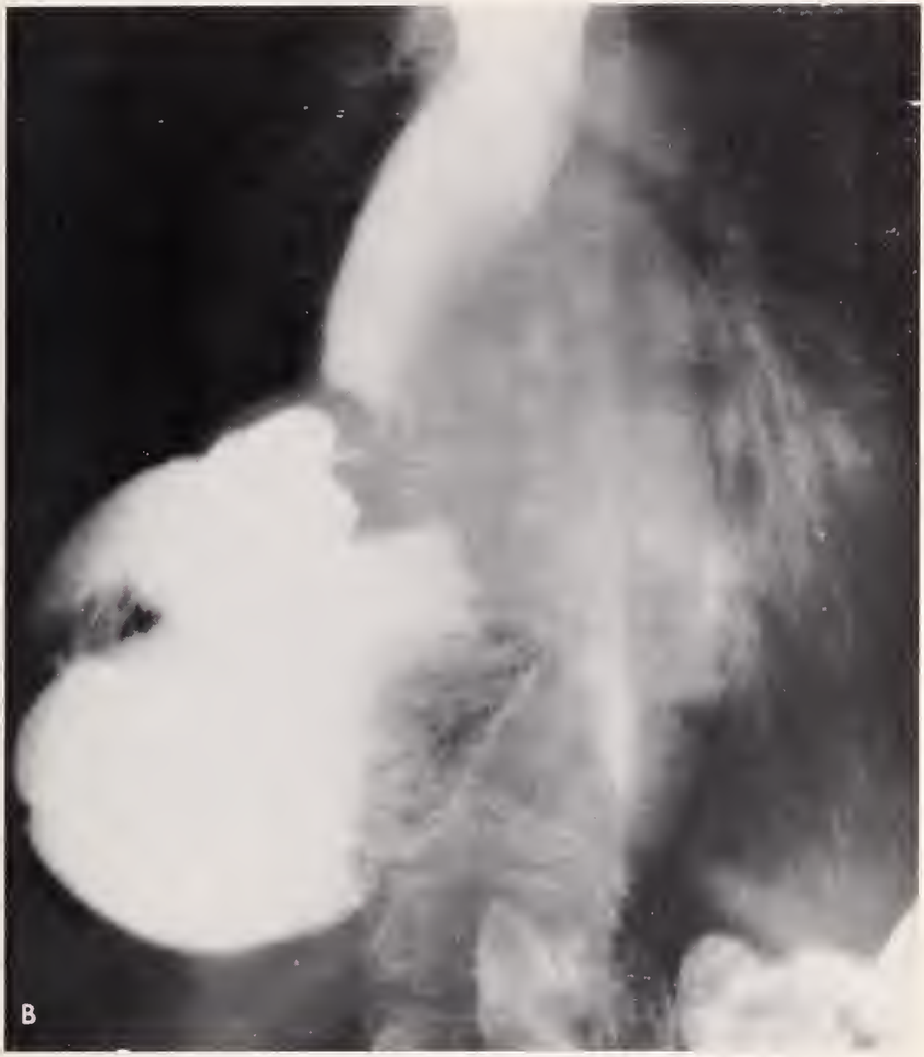
Case No. 343, Fig. 1A Postero-anterior view of the chest reveals a mass in the righ cardiophrenic region. Within the outer portion of this lesion, there is a faintly noticeable calcification (along arrows). Between the wall of the mass and the calcification, there is evidence of air, probably within a hollow viscus. The remainder of the heart and lung is normal.



Case No. 343, Fig. 1B In the lateral view, the calcifications are noted to be posteriorly located (along arrows), and the gas bubble is anterior to it.



Case No. 343, Fig. 2A Barium meal examination reveals a small amount of contrast medium in the lower esophagus (A). Mottled coarse calcifications are seen posteriorly and superiorly above the herniated portion of the stomach (B). The esophagogastric junction is noted to be considerably above the diaphragm (C). The herniated portion of the stomach is seen filled with barium (D).



Case No. 343, Fig. 2B In the supine projection, the large hernia is noted to be to the right of the midline. The calcification is not apparent because of the overlying barium within the herniated portion of the stomach.

was operated through a thoracoabdominal incision. A 3 × 5 cm calcified encapsulated smooth mass without gross overlying ulcerations was noted. This was easily shelled out. Microscopic diagnosis was leiomyoma of the esophagus.

Discussion

Leiomyomas of the esophagus constitute the most common benign tumors of the esophagus. They are most frequently located in the lower one-third of the esophagus and occur more commonly in males than females (1). They are seldom the cause of severe local symptoms, but may cause some dysphagia if the intraluminal component is large enough. The largest series of cases was reported by Story, et al. (2) In 1956 he reviewed the world literature and found 236 leiomyomas, of which 110 were operated upon. Very few cases of esophageal myomas have been noted to contain calcifications (3). When calcifications are present, they appear to be coarse and well formed, similar to those found in muscle tumors of the remainder of the upper gastrointestinal tract or uterus. The unusual aspect of this case is its presentation as a calcified mass seen on a chest x-ray next to a hollow viscus, namely the herniated portion of the stomach.

Acknowledgment

The editors wish to thank Dr. Arthur Sicular for permission to publish this case.

Case Report: LEIOMYOMA OF THE DISTAL ESOPHAGUS.

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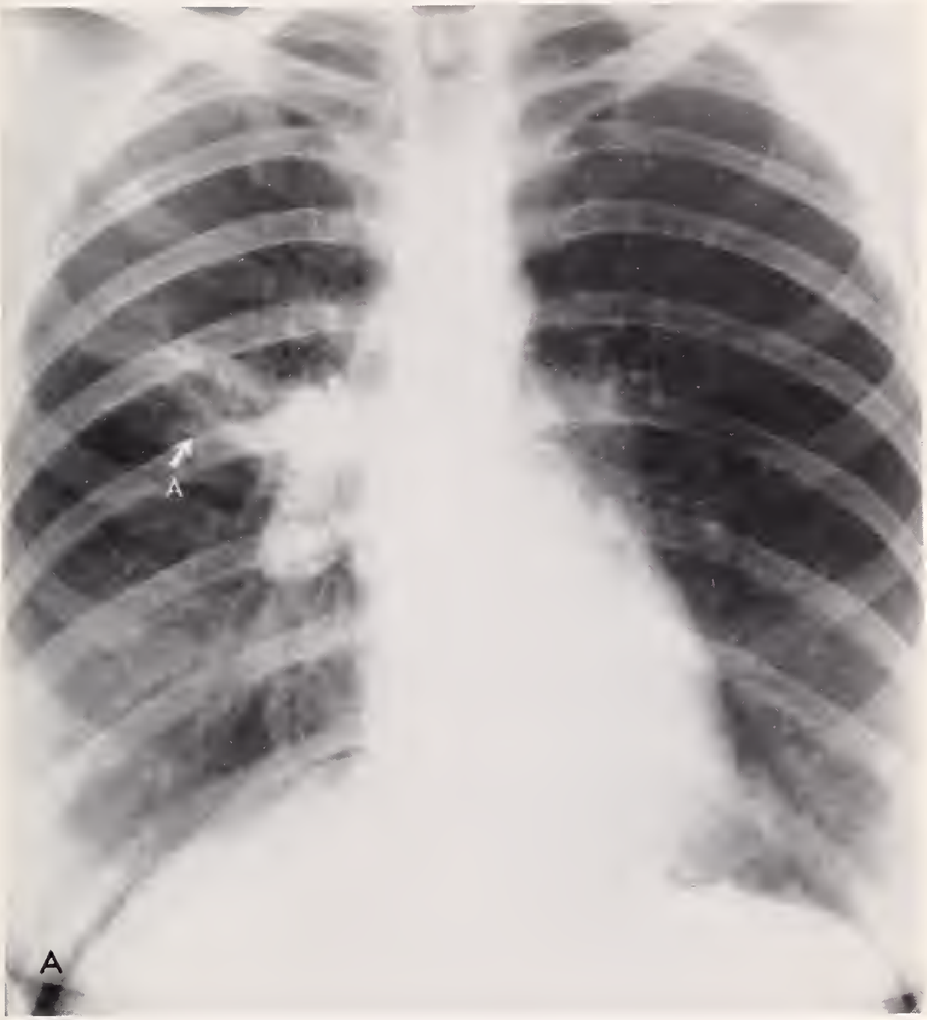
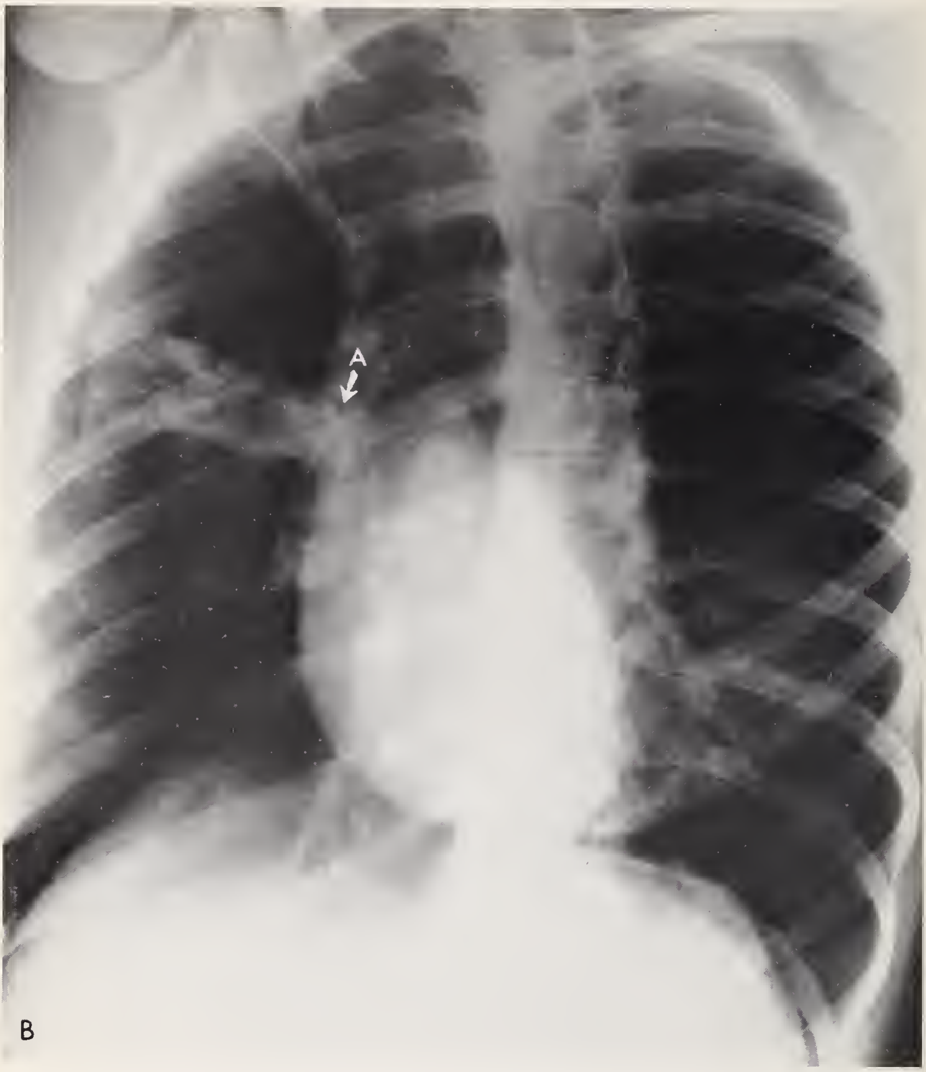
CASE NO. 344

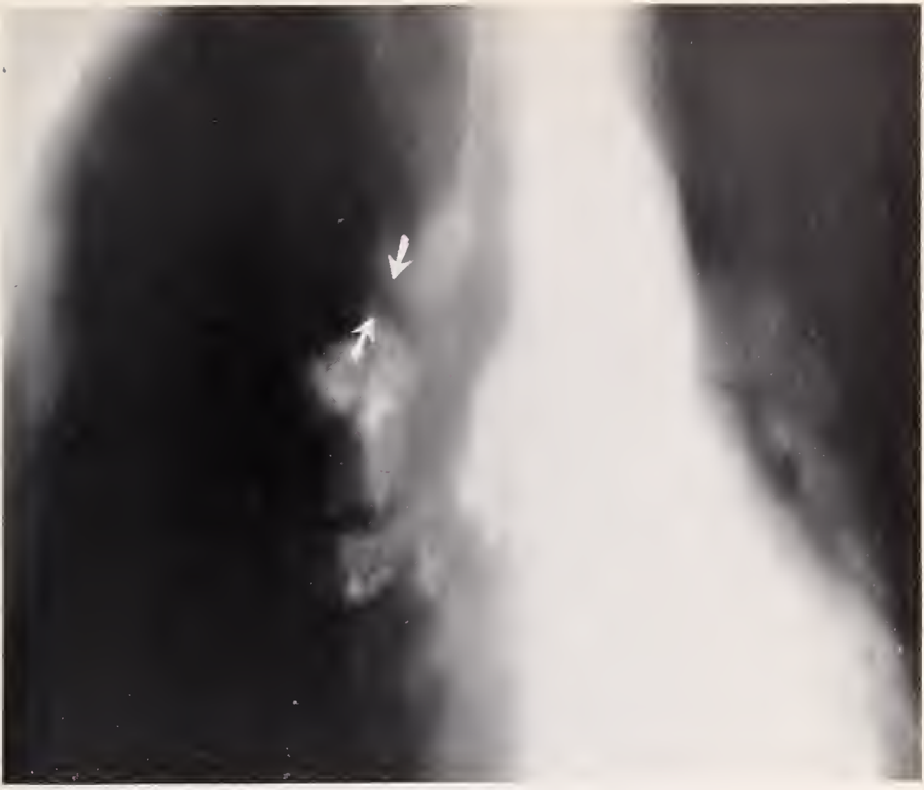
This 39-year-old woman complained of right upper chest discomfort and fever of one week's duration. There was no hemoptysis. The patient was born and raised in Florida and had been well all her life. Physical examination revealed a persistent rhonchus over the right upper lobe. Chest x-ray revealed a large mottled calcification within the right hilum. The calcifications were coarse with an irregular internal pattern. A linear mottled infiltration was noted peripherally within the right upper lobe arising from the right hilum. This wedge-shaped infiltrate extended above the minor fissure which appears to be elevated. The calcifications at the hilum were noted to be adjacent to the origin of the right upper lobe bronchus (Fig. 1). Tomographic sections were then performed with special attention to the tracheobronchial tree. These confirmed the presence of a group of coarsely calcified nodes narrowing the origin of the right upper lobe bronchus. One of the calcified deposits seems to overlie the lumen of the right upper lobe bronchus (Fig. 2).



Case No. 344, Fig. 1A There is a large mottled calcification within the right hilum as seen in the frontal view of the chest. A linear mottled infiltration is noted peripherally to the upper portion of the right hilum. There is elevation of the minor fissure (A) and tenting of the right leaf of the diaphragm. The remainder of the lung fields appear normal.



Case No. 344, Fig. 1B In the right anterior oblique view, the wedge-shaped infiltration is noted to occupy the inferior portion of the right upper lobe. A group of small calcifications are seen at the take-off of the right upper lobe bronchus (A). Other large calcified nodes are noted more inferiorly and posteriorly within the right hilum.



Case No. 344, Fig. 2 Tomographic section of the mediastinum and lung roots confirm the presence of a large group of nodes narrowing the right upper lobe bronchus (between arrows), and protruding into its lumen. The calcified nodes have a typical "popcorn" appearance. The remainder of the bronchial tree is normal.

The patient was treated with Tetracycline and all symptoms subsided. Her follow-up chest x-ray revealed some clearing of the right upper lobe infiltration with persistence of the calcified nodes at the origin of the right upper lobe bronchus. Skin tests were strongly positive to histoplasmin and negative to tuberculin and coccidioidin.

Discussion

The pathogenesis of pulmonary histoplasmosis is similar in type to that of tuberculosis. There is resolution of the primary infection which is then followed in two to five years with calcification of the primary pulmonary focus and its regional hilar nodes. The calcifications within the nodes in the hilar region in histoplasmosis are typically coarse and regular. They have been described as having a "popcorn" pattern, which is thought by some to be almost pathognomonic (1). The nodes are usually large and lobulated, and these calcified nodes are more likely to erode into the bronchus than tuberculous nodes. In a study of the etiology of broncholithiasis, Weed has cultured and isolated histoplasma capsulatum in five out of nine cases which came to thoracotomy and/or autopsy. Two additional cases showed nocardiosis. A total of nine cases were thus studied (2). In endemic areas of histoplasmosis, most calcified hilar complexes are on the basis of histoplasma capsulatum and not tuberculosis. Felson has described a thin rim of air surrounding the intrabronchial calcification giving it an unusual degree of sharpness (3). This was noted on our tomographic sections.

Acknowledgment

The editors wish to thank Dr. Alvin S. Teirstein for permission to publish this case.

Case Report: BRONCHOLITHIASIS DUE TO HISTOPLASMOSIS.

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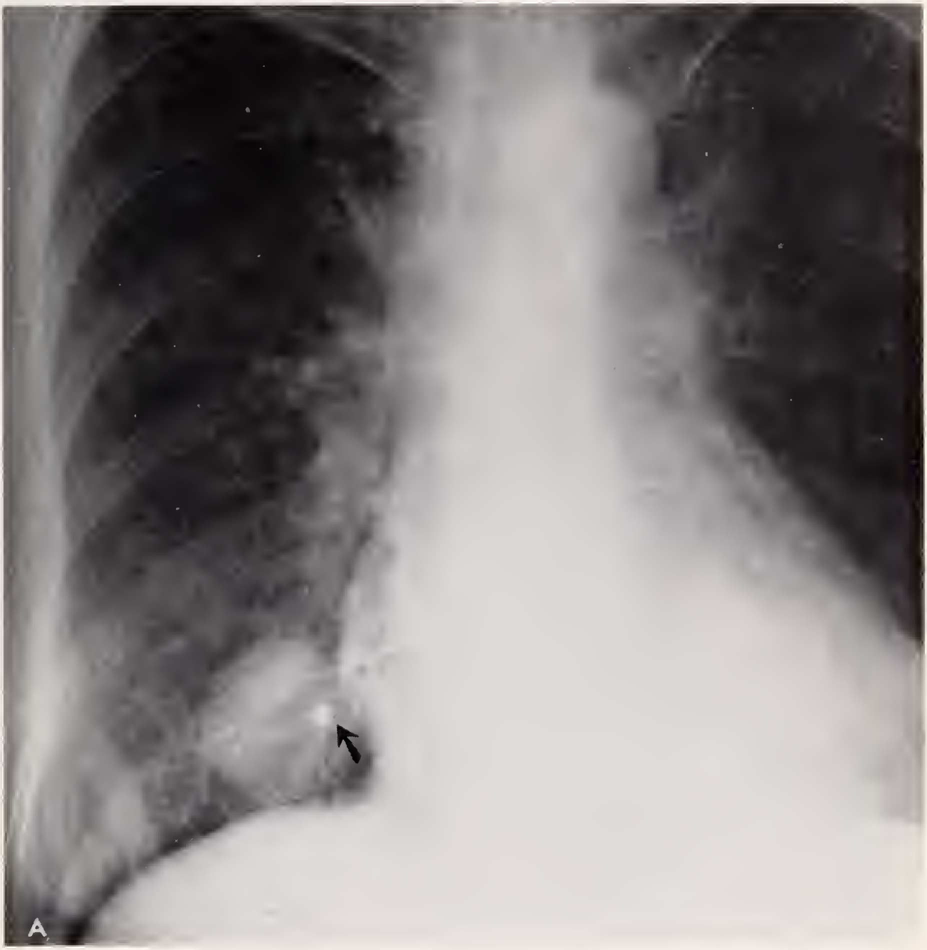
CASE NO. 345

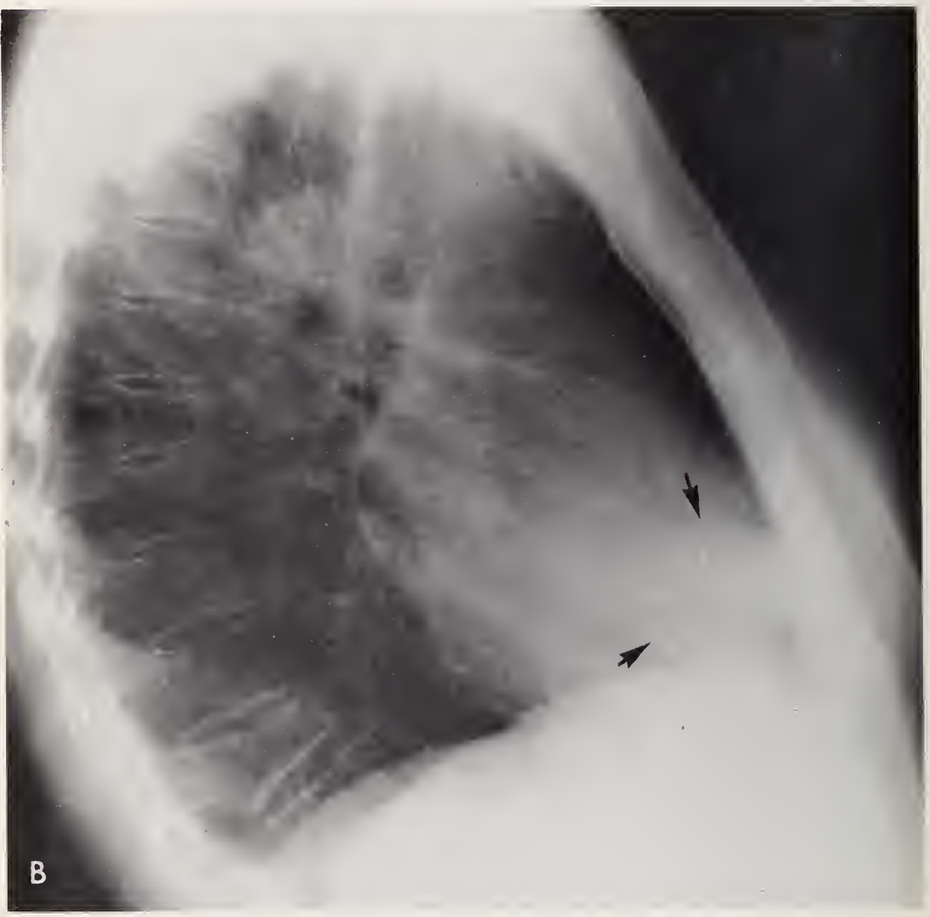
This 56-year-old woman was admitted to the hospital with a 2-week history of chest pain. She had a long history of asthmatic bronchitis and had been a heavy smoker for many years. Chest x-ray revealed a 3 cm well circumscribed rounded mass in the inferior portion of the right lung within the middle lobe (Fig. 1). A small calcification was noted to overlie its medial contour. This was confirmed on tomographic sections (Fig. 2A). However, when the patient was placed in a mild anterior oblique projection, the calcification was seen to be outside the mass and not within it (Fig. 2B). A small notching was noted in the lateral margin of this mass. Bronchoscopy was normal. Mediastinoscopy revealed a right superior mediastinal node which contained metastatic adenocarcinoma. The patient was deemed inoperable, and was given radiotherapy both to the primary lesion in the right middle lobe and to the mediastinum. Her course was progressively downhill, without any response to chemotherapy or radiotherapy and she died ten months after the initial diagnosis.

Discussion

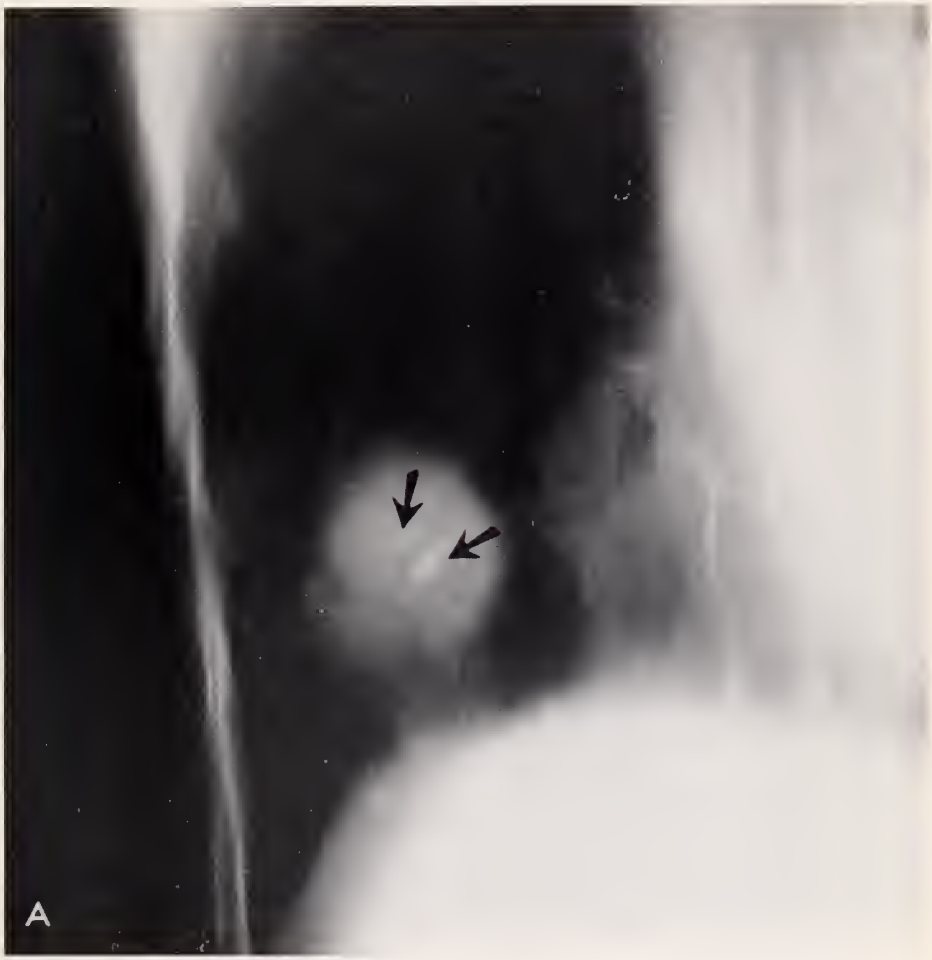
This case is of radiographic interest, because of the calcifications seen both on plain and on tomographic films overlying a sharply demarcated pulmonary mass. Usually this implies benignity. However, when the patient was rotated, in tomographic views the calcification was noted to overlie the mass and not to be within it. This is of great importance in diagnostic accuracy. It is important to realize that in any tomographic study, calcifications can appear to be in the same plane, if they are either dense enough or large enough in relation to the lesion. Careful scrutiny of the plain films and rotation of the patient into



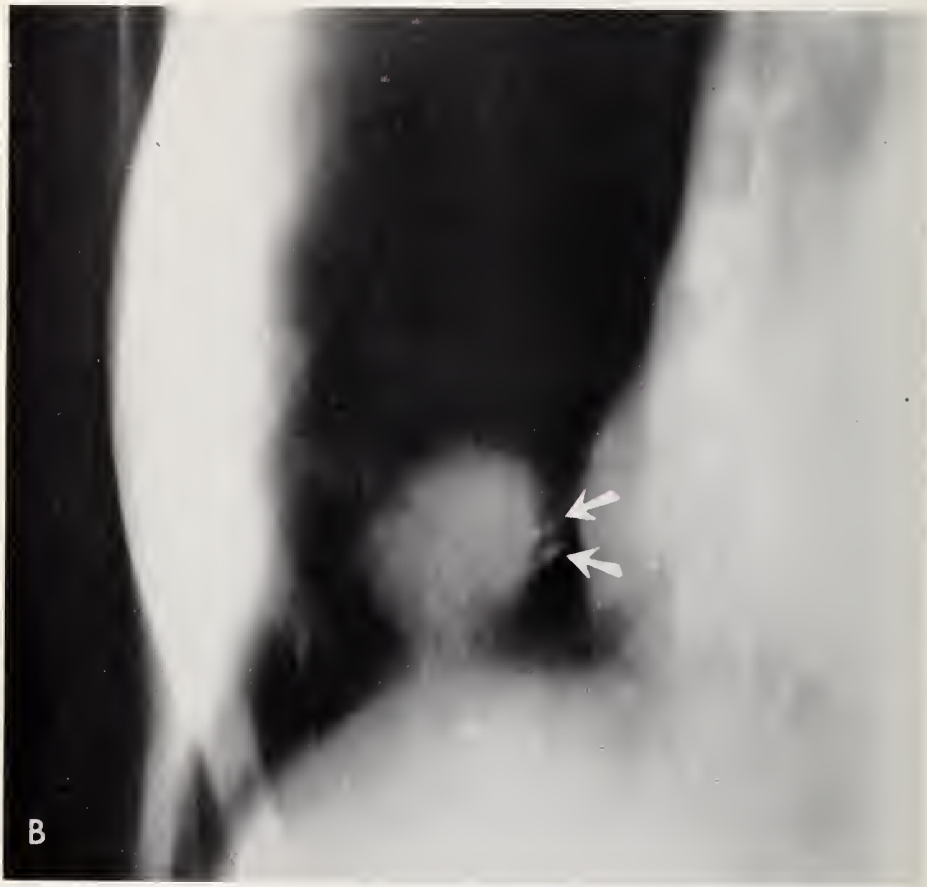
Case No. 345, Fig. 1A Examination of the chest in the postero-anterior view reveals a large rounded density in the inferior and medial portion of right lung, measuring 3 cm. in diameter. A small calcification is noted overlying its medial contour (arrow). The remainder of the lung fields appear normal.



Case No. 345, Fig. 1B Examination of the chest in the lateral view reveals the mass lesion to be situated anteriorly within the middle lobe (between arrows). The calcification noted in the frontal view is not identified.



Case No. 345, Fig. 2A Tomographic section in the postero-anterior projection reveals the lesion to be sharply circumscribed superiorly and medially. A small notching is noted upon its lateral margin. A linear area of peripheral atelectasis is noted inferiorly. Two sharply demarcated coarse calcifications are noted overlying the center of the lesion (arrow).



Case No. 345, Fig. 2B Repeat tomography with the patient placed in a mild anterior oblique projection again reveals the lesion described in the inferior portion of the right middle lobe. In this oblique tomogram, taken at the same level as Fig. 2A, the two calcifications are noted to be in a different plane than the mass lesion and, therefore, not within the mass (arrows).

various obliquities are therefore necessary before a calcification can be certified to be within a mass lesion in the chest.

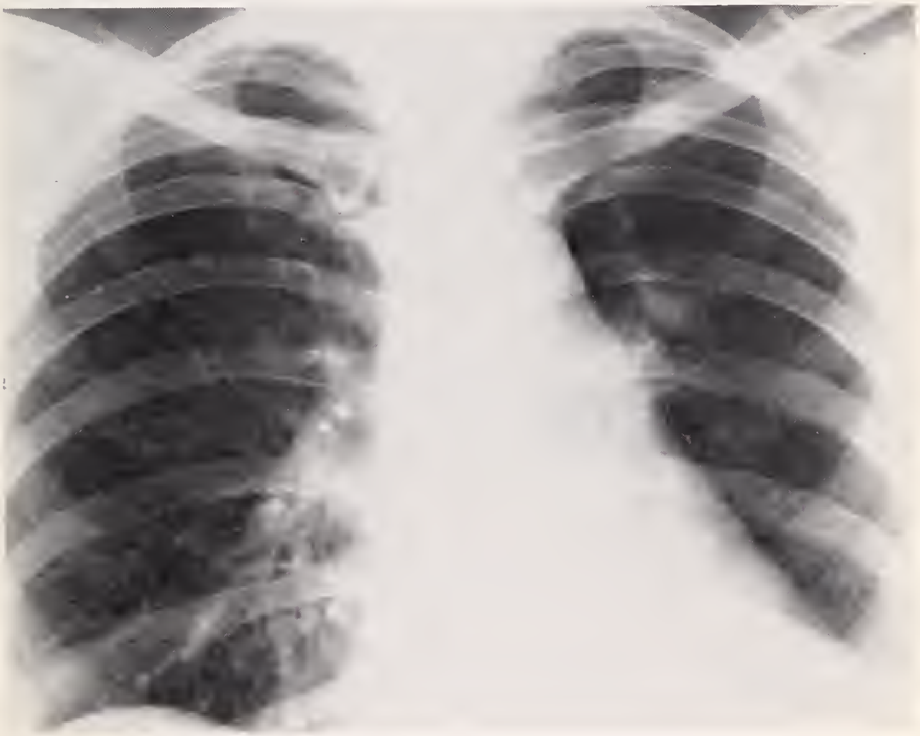
Acknowledgment

The editors wish to thank Dr. Alvin S. Teirstein and Dr. Paul A. Kirschner for permission to publish this case.

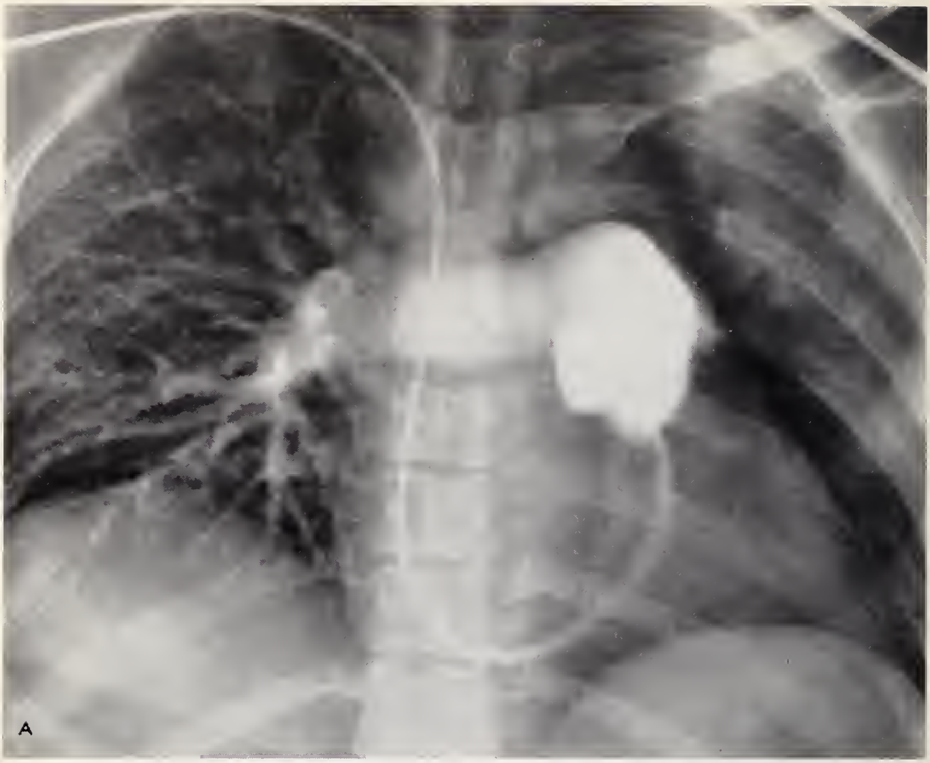
Case Report: CARCINOMA OF THE LUNG—RIGHT MIDDLE LOBE

CASE NO. 346

This 39-year-old woman had a fever of unknown etiology for over one year associated with weight loss, but she had no chest pain or cough. Initial radiographs of the chest revealed a faintly demarcated infiltration of the left upper lung field, adjacent to the left lung root. There was a subtle but definite asymmetry in the prominence of the pulmonary vascular markings, with the



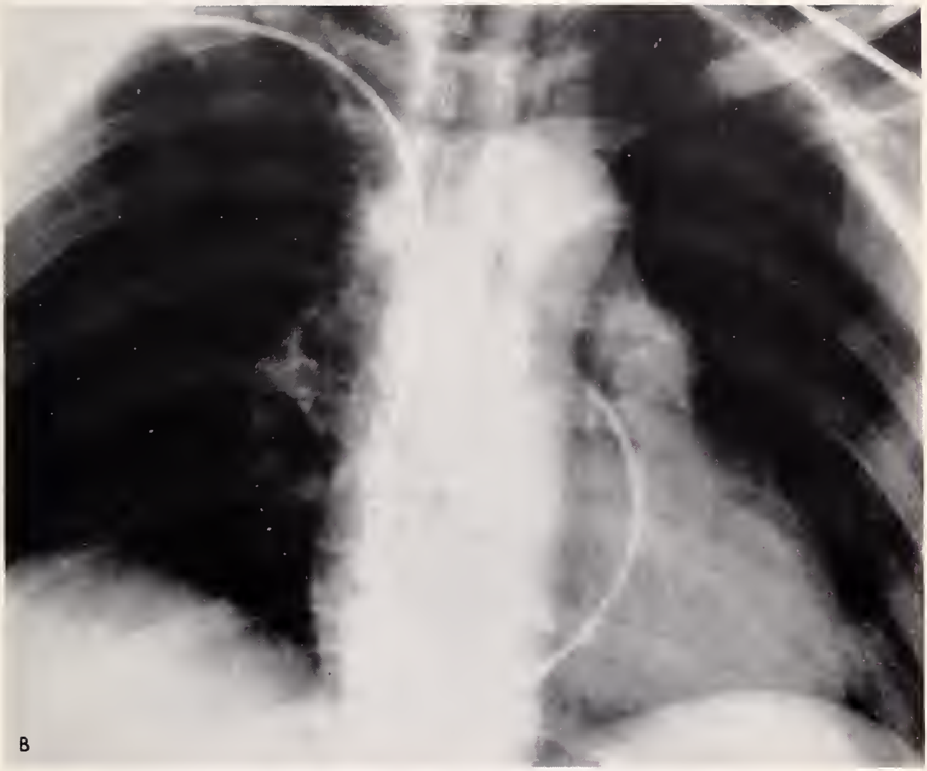
Case No. 346, Fig. 1 Examination of the chest in the frontal view (November 1968) reveals a faint infiltration in the left upper lung field adjacent to the left hilum. There is an asymmetry of the pulmonary markings with marked sparsity on the left side as compared with the right, especially in the upper two-thirds of the lung field. The heart and mediastinum appear normal. The right lung root appears normal. There is absence of the normal expected density of the left root vessels.



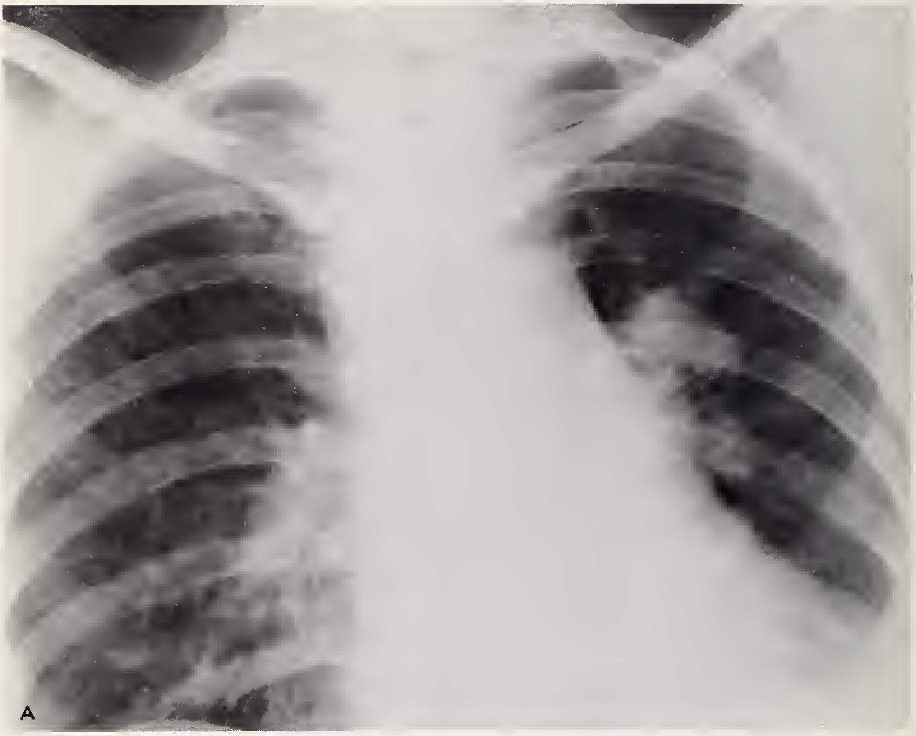
Case No. 346, Fig. 2A Selective angiography of the main pulmonary artery reveals good visualization of the pulmonary valve, main pulmonary artery, right pulmonary artery and its branches. There is no evidence of any contrast medium entering the left pulmonary artery or its branches.

right being normal and the left being relatively avascular (Fig. 1A). The left lung root was small as compared with the right side. The patient was admitted to the hospital, and selective angiography of the pulmonary artery was performed. There was good visualization of the pulmonary valve and the main pulmonary artery, as well as the right main pulmonary artery and its branches. On the left side, there was a sharp cutoff, and no contrast medium entered the left pulmonary artery or its branches (Fig. 2A). In a later film of the same sequence, there was evidence of opacification of the bronchial arteries on the left side (Fig. 2B). Mediastinoscopy and supraclavicular lymph node biopsies revealed no malignancy. Follow-up radiographs of the chest revealed an enlargement of the left hilar shadow, with a sharply demarcated density within the region of the left main pulmonary artery. This same parahilar configuration remained unchanged over the next 18 months. The patient became asymptomatic after being placed on steroid therapy.

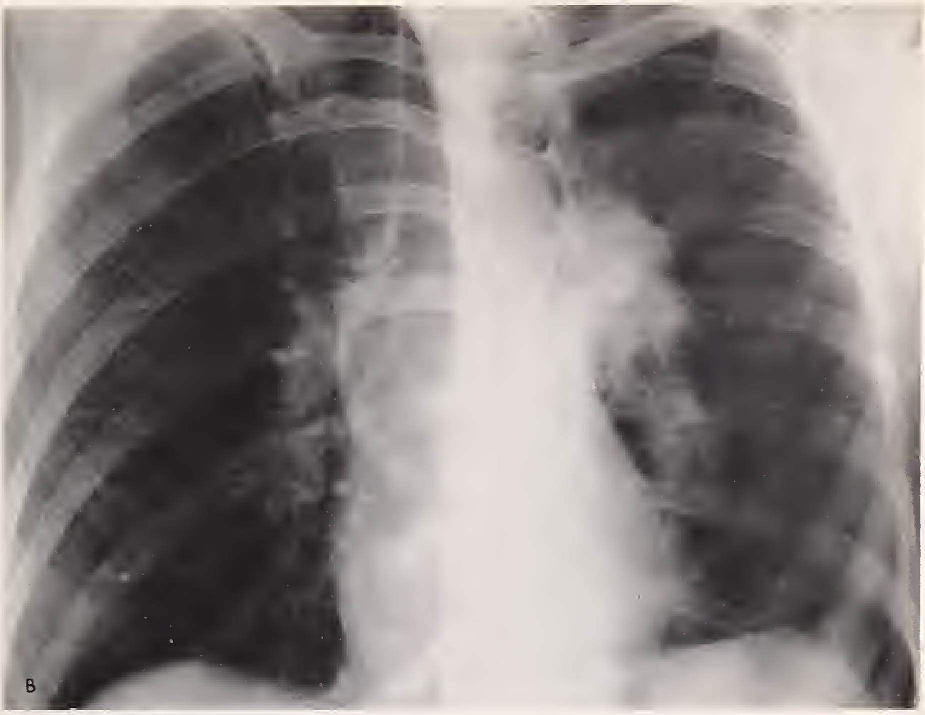
This case is of particular interest, in that it demonstrates changes in the



Case No. 346, Fig. 2B A later film during the same sequence reveals opacification of small irregular vessels in the lower two-thirds of the left lung. These undoubtedly represent enlarged bronchial arteries within the left lung.



Case No. 346, Fig. 3A Examination of the chest in the frontal view (August 1969) reveals a sharply demarcated lobulated density at the left hilum, again with almost complete absence of the normal vascular markings in the left lung. This parahilar density is larger and denser than in Fig. 1A. This pseudotumor undoubtedly represents a thrombus within the lumen of the left main pulmonary artery.



Case No. 346, Fig. 3B, The remarkable appearance of the left lung root is better appreciated with the patient in the left antierior oblique projection. The thrombosed pulmonary vessel is dense and sharply outlined by the relatively hyperlucent lung.

appearance of the thrombosed left pulmonary artery over a year and a half period. The occluded main pulmonary branch was beautifully demonstrated with direct angiographic studies. The differential diagnosis rests between a primary thrombotic phenomenon causing the occlusion, and tumor at the left hilum causing invasion and secondary occlusion of the main vessel. In this case mediastinoscopy and follow-up therapy without specific antitumerous therapy showed no evidence of malignancy.

Acknowledgment

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Case Report: OCCLUSION OF THE LEFT MAIN PULMONARY ARTERY.

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